IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 99-372-F1)

In re Application of: Welcher et al.	)	
	)	
Serial No.: 11/200,389	)	**Before the Examiner: J. Seharaseyon**
	)	
Filed: August 8, 2005	)	**Group Art Unit: 1647**
	)	
For: Interferon-like Molecules and Uses Thereof	)	**Confirmation No.: 3469**
	)	

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

RESPONSE TO OFFICE ACTION MAILED NOVEMBER 23, 2007

Responsive to the Office Action mailed November 23, 2007, Applicants respectfully request reconsideration of the above-identified application in view of the following amendments and remarks.

Amendments to the Specification: Pursuant to 37 C.F.R. § 1.121, Applicants present the amendments to the specification at page 2, marked up to show changes made relative to the immediate prior version of the specification.

Amendments to the Claims: Pursuant to 37 C.F.R. § 1.121, Applicants present a complete listing of the claims, including marked up versions of all currently amended claims, at pages 3-5

Remarks: Applicants' Remarks begin on page 6 of this paper.

Amendments to the Specification under 37 C.F.R. § 1.121

Please amend the specification at page 1, line 1 as follows:

INTERFERON-LIKE ~~MOLECULES~~ PROTEINS AND USES THEREOF

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Amendments to the Claims under 37 C.F.R. § 1.121

Claim 1 (original): An isolated polypeptide comprising an amino acid sequence:

(a) as set forth in SEQ ID NO: 5; or

(b) encoded by the DNA insert in ATCC Deposit No. PTA-976.

Claim 2 (currently amended): An isolated polypeptide comprising~~:~~

~~(a) an~~ the amino acid sequence as set forth in SEQ ID NO: 6, optionally further comprising an amino-terminal methionine~~;~~

~~(b) an amino acid sequence that is at least about 70 percent identical to the amino acid sequence set forth in SEQ ID NO: 5, wherein the polypeptide upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation; or~~

~~(c) a fragment of the amino acid sequence set forth in SEQ ID NO: 5 comprising at least about 25 amino acid residues, wherein the polypeptide upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation, or is antigenic~~.

Claim 3 (cancelled).

Claim 4 (currently amended): An isolated polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence:

(a) as set forth in SEQ ID NO: 4;

(b) of the DNA insert in ATCC Deposit No. PTA-976; or

(c) encoding a polypeptide as set forth in SEQ ID NO: 5~~; or~~

~~(d) that hybridizes to the complement of the nucleotide sequence of any of (a)-(c) under hybridization conditions allowing no more than a 21% mismatch between the nucleotide sequences;~~

~~wherein the encoded polypeptide upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation~~.

Claims 5-7 (cancelled).

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Claim 8 (currently amended): A composition comprising the polypeptide of any of Claims 1, 2, or ~~3~~ 4, and a pharmaceutically acceptable formulation agent.

Claim 9 (original): The composition of Claim 8, wherein the pharmaceutically acceptable formulation agent is a carrier, adjuvant, solubilizer, stabilizer, or anti-oxidant.

Claim 10 (currently amended): The composition of Claim 8, wherein the polypeptide comprises ~~an~~ the amino acid sequence as set forth in SEQ ID NO: 6.

Claim 11 (currently amended): A polypeptide comprising a derivative of the polypeptide of any of Claims 1, 2, or ~~3~~ 4.

Claim 12 (original): The polypeptide of Claim 11 that is covalently modified with a water-soluble polymer.

Claim 13 (original): The polypeptide of Claim 12, wherein the water-soluble polymer is polyethylene glycol, monomethoxy-polyethylene glycol, dextran, cellulose, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxidelethylene oxide copolymers, polyoxyethylated polyols, or polyvinyl alcohol.

Claim 14 (currently amended): A fusion polypeptide comprising the polypeptide of any of Claims 1, 2, or ~~3~~ 4 fused to a heterologous amino acid sequence.

Claim 15 (original): The fusion polypeptide of Claim 14, wherein the heterologous amino acid sequence is an IgG constant domain or fragment thereof.

Claim 16 (currently amended): A polypeptide produced by a process comprising culturing a host cell comprising a vector comprising a nucleic acid molecule comprising a nucleotide sequence:

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(a) as set forth in SEQ ID NO: 4;

(b) of the DNA insert in ATCC Deposit No. PTA-976; or

(c) encoding a polypeptide as set forth in SEQ ID NO: 5; ~~or~~

~~(d) that hybridizes to the complement of the nucleotide sequence of any of (a)-(c) under hybridization conditions allowing no more than a 21% mismatch between the nucleotide sequences,~~

~~wherein the polypeptide upon exposure to mammalian cells, causes an increase in cellular protein tyrosine phosphorylation;~~

under suitable conditions to express the polypeptide, and optionally isolating the polypeptide from the culture.

Claims 17-18 (cancelled).

Claim 19 (currently amended): The polypeptide of ~~any of~~ Claim[[s]] 16~~, 17, or 18~~, wherein the host cell is a eukaryotic cell.

Claim 20 (currently amended): The polypeptide of ~~any of~~ Claim[[s]] 16~~, 17, or 18~~, wherein the host cell is a prokaryotic cell.

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REMARKS

Claims 2, 4, 8, 10, 11, 14, 16, 19, and 20, as amended, and claims 1, 9, 12, 13, and 15 are pending in the instant application. Claims 3, 5-7, 17, and 18 have been canceled without prejudice or disclaimer. No new matter has been added as a result of the above-described amendments. The rejections set forth in the Office Action have been overcome by amendment or are traversed by argument below.

1. Claim of priority

The Office Action states that the instant application appears to claim subject matter disclosed in U.S. Application No. 09/927,850 (the '850 application), and that Applicants must amend the specification to insert a reference to the '850 application as the first sentence of the specification if Applicants intend to rely on the filing date of the '850 application under 35 U.S.C. §§ 119(e), 120, 121, or 365(c).

Coinciding with the submission of this Response, Applicants have submitted a Petition for an Unintentionally Delayed Domestic Priority Claim in order to amend the first sentence of the instant application to insert a reference to the '850 application.

2. Objections to the Specification

The Office Action contains an objection to the specification because the title of the invention is not descriptive. The Action states that a new title is required that is clearly indicative of the invention to which the claims are directed.

Applicants have amended the title to read: "Interferon-Like Proteins and Uses Thereof," which Applicants contend is clearly indicative of the invention to which the claims are directed. Applicants, therefore, respectfully request that this ground of objection be withdrawn.

The Office Action asserts an objection to the specification because of the improper use of the trademark "Quick Spin" and "Taq polymerase," which the Action states should be capitalized and accompanied by generic terminology.

Applicants have searched the U.S. Patent and Trademark Office Trademark Electronic Search System (TESS), but have not found a record for the term "Quick Spin" with respect to

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Qiagen's G-50 column. Applicants were also unable to find a record for the term "Taq polymerase." Applicants contend that because the terms "Quick Spin" and "Taq polymerase" do not appear to be registered trademarks, these terms do not to be capitalized or accompanied by generic terminology. Applicants respectfully request that this ground of objection be withdrawn.

3. Rejection of claims 5, 6, and 16-20 under 35 U.S.C. § 112, second paragraph

Claims 5, 6, and 16-20 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that applicants regard as the invention.

a. Hybridization conditions

Claims 5, 6, and 16-18 are rejected for reciting the term "hybridization conditions," which the Action states is insufficiently defined in the specification.

Applicants have cancelled claims 5, 6, 17, and 18, and have amended claim 16 to delete subpart (d). As claim 16 no longer recites hybridization conditions, Applicants respectfully request that this ground of rejection be withdrawn.

b. Polypeptide production

Claims 16-18 are rejected for being indefinite since it is unclear how the polynucleotide complements of claims 16(d), 17(c), and 18(b) can encode a polypeptide having the activity of the polypeptides disclosed in the specification.

Applicants have cancelled claims 17 and 18, and have amended claim 16 to delete subpart (d). As claim 16 no longer recites subpart (d), Applicants respectfully request that this ground of rejection be withdrawn.

c. IFN-L polypeptide

Claims 2, 5, and 17 are rejected as being vague and indefinite for the reciting the term "at least about."

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Applicants have cancelled claims 5 and 17, and have amended claim 2 to delete subpart (b). As claim 2 no longer recites the term "about," Applicants respectfully request that this ground of rejection be withdrawn.

Applicants submit that the claims as amended are in condition for allowance and respectfully request that the rejections under 35 U.S.C. § 112, second paragraph, be withdrawn.

4. Rejection of claims 1-20 under 35 U.S.C. § 112, first paragraph

a. Rejection of claims 1-20 under the enablement requirement of 35 U.S.C. § 112, first paragraph

Claims 1-20 are rejected under 35 U.S.C. § 112, first paragraph, as not being enabled by the specification such that one of skill in the art could make and use the invention as claimed. The Action specifically asserts that all possible variants of SEQ ID NO: 5 are not enabled, and that the amount of experimentation required to determine these variants would be undue.

Applicants respectfully disagree with the Action's assertion that the claimed variants of SEQ ID NO: 5 are not enabled. Nevertheless, solely in an effort to expedite prosecution of the pending claims to allowance, Applicants have cancelled claims 5, 6, 17, and 18, and have deleted claims 2(b), 2(c), 4(d), and 16(d). As the pending claims no longer recite variants of SEQ ID NO: 5, Applicants respectfully request that this ground of rejection be withdrawn.

Applicants reserve the right to pursue claims directed to the cancelled or deleted subject matter in a timely filed continuation or divisional application, or alternatively, reintroduce the cancelled or deleted subject matter in the instant application at such time as the Office indicates that the pending claims are otherwise in condition for allowance.

The Action also asserts that phrases such as "an amino acid sequence" and "a nucleic acid molecule" in the claims read upon various variants and fragments. Applicants have cancelled claim 6 and 18, and have amended claims 2(a) and 10 to recite "the" rather than "a" or "an." Applicants, therefore, respectfully request that this ground of rejection be withdrawn.

The Action further asserts that Applicants' referral to the deposit of PTA-976 in the specification and in claims 1, 4, 5, 16 and 17 is an insufficient assurance that all of the conditions of 37 C.F.R §§ 1.801-1.809 have been met. The Action states that Applicants must submit a statement

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by an attorney of record over his or her signature, stating that a deposit has been made under the terms of the Budapest Treaty and that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent. The Action further states that the instant specification must be amended to recite the date of the deposit and the complete name and address of the depository, and that the claims must be amended to recite the accession number.

Pursuant to the Examiner's request, Applicants' representative submits the following statement: Applicants deposited cDNA encoding human IFN-L polypeptide with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209. The deposit was accepted by the ATCC, an International Depository Authority, under the provisions of the Budapest Treaty, and the deposit was designated as PTA-976. A copy of the ATCC receipt for this deposit, showing the patent deposit designation (Accession No. PTA-976) and the date on which the deposit was received by the ATCC (November 23, 1999), is attached. Pursuant to 37 C.F.R. § 1.808(a)(2), the deposit was made under conditions that assure that all restrictions imposed by the depositors on the availability to the public of the deposited material would be irrevocably removed upon the granting of a patent relying on the deposited biological material. In making the deposit, Applicants acknowledged their responsibility, pursuant to 37 C.F.R. § 1.805, to provide a replacement or supplemental deposit if the depository possessing the deposit is unable to furnish samples thereof or is able to furnish samples thereof but the deposit has become contaminated or has lost its capability to function as described in the specification. With regard to the assertion that the date of the deposit and the complete name and address of the depository is not referred to in the body of the specification, Applicants respectfully direct the Examiner's attention to page 92, lines 25-28 of the specification as-filed, where Applicants disclose that a deposit of cDNA encoding human IFN-L polypeptide, subcloned into pSPORT1 (Gibco BRL) and transfected into *E. coli* strain DH10B, having Accession No. PTA-976, were made with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on November 23, 1999. With regard to the assertion that the accession number of the deposit is not referred to in the claims, Applicants respectfully direct the Examiner's attention to claims 1(b), 4(b), and 16(b), as originally filed. Applicants contend that all the requirements of 37 C.F.R. §§ 1.801-1.809 have been met. *In re*

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Lundak, 225 U.S.P.Q. 90 (Fed. Cir. 1985). Withdrawal of this rejection is therefore respectfully solicited.

Applicants submit that the claims as amended are in condition for allowance, and respectfully request that the rejections under the enablement requirement of 35 U.S.C. § 112, first paragraph, be withdrawn.

b. Rejection of claims 1-20 under the written description requirement of 35 U.S.C. § 112, first paragraph

Claims 1-20 have been rejected as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Action asserts that the specification does not disclose all possible variants of nucleic acid molecules that hybridize to the complement of the claimed nucleotide sequences with more than 21% mismatch. The Action also asserts that specification has failed to disclose any other sequence contemplated in the instant claims including IFN-L fragments and other variants, and thus the skilled artisan cannot envision the detailed chemical structures of the claimed polypeptide sequences. Finally, the Action asserts that the species specifically disclosed are not representative of the genus because the genus is highly variant.

Applicants respectfully disagree with the Action's assertion that the specification does not contain an adequate written description of the claimed invention. Nevertheless, solely in an effort to expedite prosecution of the pending claims to allowance, Applicants have cancelled claims 5, 6, 17, and 18, and have deleted claims 2(b), 2(c), 4(d), and 16(d). As the pending claims no longer recite the variants described above, Applicants respectfully request that this ground of rejection be withdrawn.

Applicants reserve the right to pursue claims directed to the cancelled or deleted subject matter in a timely filed continuation or divisional application, or alternatively, reintroduce the cancelled or deleted subject matter in the instant application at such time as the Office indicates that the pending claims are otherwise in condition for allowance.

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Applicants submit that the claims as amended are in condition for allowance, and respectfully request that the rejection under the written description requirement of 35 U.S.C. § 112, first paragraph, be withdrawn.

5. Rejection of claims 1-20 under 35 U.S.C. § 102(e)

The Office Action asserts a rejection of claims 1-20 under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,433,145 (the '145 patent).

Pursuant to 37 C.F.R. § 41.202, Applicants suggest that an interference be declared between the instant application and U.S. Patent No. 6,433,145 (the '145 patent). The '145 patent issued on August 13, 2002 from U.S. Application No. 09/487,792 (the '792 application), which was filed on January 20, 2000. The '792 application is a continuation-in-part of U.S. Application No. 09/358,587 and International Application No. PCT/US99/16424, both filed on July 21, 1999, and claims the benefit of U.S. Provisional Application No. 60/093,643, filed July 21, 1998.

Applicants believe claims 1 and 51 of the '145 patent interfere with claims 1 and 2 of the instant application. Claims 1 and 51 of the '145 patent read as follows:

> 1. An isolated protein comprising a polypeptide having an amino acid sequence selected from the group consisting of:
> (a) amino acids 1 to 207 of SEQ ID NO:2;
> (b) amino acids 7 to 207 of SEQ ID NO:2;
> (c) amino acids 2 to 207 of SEQ ID NO:2; and
> (d) amino acids 28 to 207 of SEQ ID NO:2.
>
> 51. An isolated protein comprising a polypeptide having an amino acid sequence selected from the group consisting of:
> (a) the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment has anti-viral activity;
> (b) the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment inhibits bone marrow proliferation;
> (c) the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment activates the Jak/Stat pathway; and
> (d) the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment binds an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO:2.

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Claims 1 and 2 of the instant application read as follows:

1. An isolated polypeptide comprising an amino acid sequence:
   (a) as set forth in SEQ ID NO: 5; or
   (b) encoded by the DNA insert in ATCC Deposit No. PTA-976.

2. An isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 6, optionally further comprising an amino-terminal methionine.

Applicants propose the following counts:

1. An isolated polypeptide comprising the amino acid sequence of amino acids 1 to 207 of SEQ ID NO:2 of U.S. Patent No. 6,433,145.

2. An isolated polypeptide comprising the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment has anti-viral activity, inhibits bone marrow proliferation, activates the Jak/Stat pathway, or binds an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO:2.

A comparison between the proposed counts, claims 1(a) and 51 of the '145 patent, and claims 1(a) and 2 of the instant application is provided in the claim chart below:

Count 1	**Claim 1(a) of '145 patent**	**Claim 1(a) of the instant application**
An isolated polypeptide	An isolated protein comprising a polypeptide	An isolated polypeptide
comprising the amino acid sequence of amino acids 1 to 207 of SEQ ID NO:2. of U.S. Patent No. 6,433,145.	having an amino acid sequence [that is] amino acids 1 to 207 of SEQ ID NO:2[.]	comprising an amino acid sequence . . . as set forth in SEQ ID NO: 5 . . . .
Count 2	**Claim 51 of '145 patent**	**Claim 2 of the instant application**
An isolated polypeptide	An isolated protein comprising a polypeptide	An isolated polypeptide
comprising the amino acid	having an amino acid	comprising the amino acid

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sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2,	sequence [that is] the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2,	sequence as set forth in SEQ ID NO: 6 . . . .
wherein the fragment has anti-viral activity, inhibits bone marrow proliferation, activates the Jak/Stat pathway, or binds an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO:2.	wherein the fragment has anti-viral activity; . . . wherein the fragment inhibits bone marrow proliferation; . . . wherein the fragment activates the Jak/Stat pathway; [or] wherein the fragment binds an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO:2.	[While claim 2 does not expressly recite this limitation, the polypeptide recited in the claim, which is the mature form of the full-length IFN-L polypeptide, would inherently have such properties.]

The sequence alignment provided in Exhibit A indicates that the amino acid sequence of SEQ ID NO: 5 of the instant application shares 100% sequence identity with amino acids 1 to 207 of SEQ ID NO: 2 of the '145 patent. Applicants contend that because the amino acid sequence of SEQ ID NO: 5 of claim 1(a) of the instant application and amino acids 1 to 207 of SEQ ID NO: 2 of claim 1(a) of the '145 patent share 100% identity, the subject matter of either claim would, if prior art, have anticipated the subject matter of the other claim.

The sequence alignment provided in Exhibit B indicates that the amino acid sequence of SEQ ID NO: 6 of the instant application shares 100% sequence identity with amino acids 30 to 207 of SEQ ID NO: 2 of the '145 patent. The amino acid sequence of SEQ ID NO: 6 is, therefore, a fragment of amino acid residues 1 to 207 of SEQ ID NO: 2 of the '145 patent. Applicants contend that because the amino acid sequence of SEQ ID NO: 6 is the mature form of the amino acid sequence of SEQ ID NO: 2 of the '145 patent (as well as of the amino acid sequence of SEQ ID NO: 5 of the instant application), the amino acid sequence of SEQ ID NO: 6 would have anti-viral

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activity, inhibit bone marrow proliferation, activate the Jak/Stat pathway, or bind an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO: 2. Applicants also contend that because the amino acid sequence of SEQ ID NO: 6 of claim 2 of the instant application shares 100% identity with a fragment of the amino sequence of SEQ ID NO: 2 of the '145 patent, and the mature form would have anti-viral activity, inhibit bone marrow proliferation, activate the Jak/Stat pathway, or bind an antibody that specifically binds a protein having the amino acid sequence of SEQ ID NO: 2, the subject matter of either claim 51 of the '145 patent and claim 2 of the instant application would, if prior art, have anticipated the subject matter of the other claim.

Applicants contend that the Declaration Pursuant to 37 C.F.R. § 1.131 which is provided in Exhibit C, and which was submitted on June 24, 2004 for U.S. Application No. 09/927,850 (from which the instant application claims the benefit of priority as a continuation application), establishes that Applicants will prevail on priority were an interference between the instant application and the '145 patent to be declared. In particular, the Declaration provides copies of forty-one (41) pages from the inventors' laboratory notebook showing conception of the claimed invention before July 21, 1998. The laboratory notebook pages show that a genomic cloning approach was used to identify the nucleic acid sequence of human interferon-like polypeptide (*see* page 34 of laboratory notebook pages). Specifically, three genomic clones were identified as containing nucleic acid sequences encoding at least a portion of human interferon-like polypeptide (clones 2, 6, and 7; *see* page 40 of laboratory notebook pages). The nucleic acid sequences from these clones were isolated and then re-cloned into a suitable sequencing vector. One of the three genomic clones was determined to contain a partial nucleic acid sequence for human interferon-like polypeptide and another genomic clone (clone 6) was determined to contain a full-length nucleic acid sequence for human interferon-like polypeptide (*see* page 62 of laboratory notebook pages). The amino acid sequence of human interferon-like polypeptide was determined from the latter nucleic acid sequence.

The Declaration also provides copies of ten (10) pages from a Research Summary prepared by the inventors showing that the invention disclosed and claimed in the instant patent application was diligently reduced to practice. Specifically, the Research Summary shows that the inventors performed experiments in order to determine the function of protein encoded by the nucleic acid sequence described above, and that once the function of the protein had been determined, the inventors prepared a Research Summary and submitted that Summary to the legal department of

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Amgen Inc., the assignee of the instant application. More particularly, the Research Summary shows that several versions of the human and rat IFN-L proteins were produced in a mammalian expression system (*see* page 7 of Research Summary) and that rat IFN-L:Fc fusion protein treatment of several cell lines was found to cause phosphorylation of cellular proteins (*see* page 10 of Research Summary). Thus, the Declaration and attachments provided in Exhibit C establish that Applicants will prevail on priority were an interference between the instant application and the '145 patent to be declared.

Applicants note that claims 1(a) and 2 of the instant application were not added or amended in order to provoke an interference. Applicants contend, therefore, that pursuant to 37 C.F.R. § 41.202(a)(5), a claim chart showing the written description for claims 1(a) and 2 in the specification need not be provided.

Finally, Applicants submit the following chart showing where the instant disclosure provides a constructive to reduction of practice within the scope of the interfering subject matter:

Count 1	**Reduction to Practice in Instant Disclosure**
An isolated polypeptide comprising the amino acid sequence of amino acids 1 to 207 of SEQ ID NO:2. of U.S. Patent No. 6,433,145.	page 4, lines 9-12; page 7, lines 28-30; page 11, lines 4-5; page 95, lines 10-16; and Figures 2A-2B.
Count 2	**Reduction to Practice in Instant Disclosure**
An isolated polypeptide comprising the amino acid sequence of a fragment of amino acid residues 1 to 207 of SEQ ID NO:2, wherein the fragment has anti-viral activity.	page 4, lines 16-19; page 12, line 28 to page 13, line 3; page 7, lines 28-30; page 95, lines 10-16; page 103, lines 20-27; and Figures 2A-2B.

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In view of the above discussion, Applicants respectfully suggest that an interference be declared between the instant application and the '145 patent.

CONCLUSIONS

Applicants respectfully contend that all conditions of patentability are met in the pending claims as amended. Allowance of the claims is thereby respectfully solicited.

If Examiner Seharaseyon believes it to be helpful, he is invited to contact the undersigned representative by telephone at 312-913-0001.

Respectfully submitted,
McDonnell Boehnen Hulbert & Berghoff LLP

Dated: December 23, 2008

By: /Donald L. Zuhn, Jr./
Donald L. Zuhn, Jr., Ph.D.
Reg. No. 48,710

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Exhibit A

```
                  10        20        30        40        50        60
                   |         |         |         |         |         |
SEQID2       MSTKPDMIQKCLWLEILMGIFIAGTLSLDCNLLNVHLRRVTWQNLRHLSSMSNSFPVECL
SEQID5       MSTKPDMIQKCLWLEILMGIFIAGTLSLDCNLLNVHLRRVTWQNLRHLSSMSNSFPVECL
             ************************************************************
Prim.cons.   MSTKPDMIQKCLWLEILMGIFIAGTLSLDCNLLNVHLRRVTWQNLRHLSSMSNSFPVECL

                  70        80        90       100       110       120
                   |         |         |         |         |         |
SEQID2       RENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ
SEQID5       RENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ
             ************************************************************
Prim.cons.   RENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ

                 130       140       150       160       170       180
                   |         |         |         |         |         |
SEQID2       QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYSD
SEQID5       QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYSD
             ************************************************************
Prim.cons.   QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYSD

                 190       200
                   |         |
SEQID2       CAWEIVRVEIRRCLYYFYKFTALFRRK
SEQID5       CAWEIVRVEIRRCLYYFYKFTALFRRK
             **************************
Prim.cons.   CAWEIVRVEIRRCLYYFYKFTALFRRK
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Exhibit B

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                    10         20         30         40         50         60
                     |          |          |          |          |          |
SEQID2       MSTKPDMIQKCLWLEILMGIFIAGTLSLDCNLLNVHLRRVTWQNLRHLSSMSNSFPVECL
SEQID6       ----------------------------CNLLNVHLRRVTWQNLRHLSSMSNSFPVECL
                                          ******************************
Prim.cons.   MSTKPDMIQKCLWLEILMGIFIAGTLSLDCNLLNVHLRRVTWQNLRHLSSMSNSFPVECL

                    70         80         90        100        110        120
                     |          |          |          |          |          |
SEQID2       RENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ
SEQID6       RENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ
             ************************************************************
Prim.cons.   RENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFSQHTFKYWKERHLKQIQIGLDQ

                   130        140        150        160        170        180
                     |          |          |          |          |          |
SEQID2       QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYSD
SEQID6       QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYSD
             ************************************************************
Prim.cons.   QAEYLNQCLEEDENENEDMKEMKENEMKPSEARVPQLSSLELRRYFHRIDNFLKEKKYSD

                   190        200
                     |          |
SEQID2       CAWEIVRVEIRRCLYYFYKFTALFRRK
SEQID6       CAWEIVRVEIRRCLYYFYKFTALFRRK
             ***************************
Prim.cons.   CAWEIVRVEIRRCLYYFYKFTALFRRK
```


McDonnell Boehnen Hulbert & Berghoff LLP
300 South Wacker Drive
Chicago, Illinois 60606
312-913-0001

Exhibit C

McDonnell Boehnen Hulbert & Berghoff LLP
300 South Wacker Drive
Chicago, Illinois 60606
312-913-0001

IN THE UNITED ST TES PATENT AND TRADEMARK OFFICE
(Case No. 99-372-F)

PATENT

In re Application of: Welcher al.

Serial No.: 09/927,850

Filed: August 10, 2001

For: Interferon-Like Molecule and Uses Thereof

Before the Examiner: J. Andres

Group Art Unit: 1646

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARA ON PURSUANT TO 37 C.F.R § 1.131

We, Andrew A. Welcher, r iding at 1175 Church Street, Ventura, California; Duanzhi Wen, residing at 3885 Campus Drive, T ousand Oaks, California; and Michael Kelley, residing at 3866 Alta Mesa Drive, Studio City, Cal ornia; hereby declare:

1. We are named co-i ventors on United States Application No. 09/927,850, filed on August 10, 2001.

2. The invention disc sed and claimed in the instant patent application was conceived in the United States by us before ly 21, 1998 and was then diligently reduced to practice.

3. Accompanying thi Declaration are photocopies of forty-one (41) pages from our laboratory notebook showing con eption of our invention before July 21, 1998. Specifically, the photocopies of our laboratory not ook show that a genomic cloning approach was used to identify the nucleic acid sequence of huma interferon-like polypeptide (*see* page 34 of laboratory notebook). Three genomic clones were ide fied as containing nucleic acid sequences encoding at least a portion of human interferon-like p lypeptide (*i.e.*, clones 2, 6, and 7; *see* page 40). The nucleic acid sequences from these clones were olated and then re-cloned into a suitable sequencing vector. One of the three genomic clones was termined to contain a partial nucleic acid sequence for human interferon-like polypeptide and a ther genomic clone (*i.e.*, clone 6) was determined to contain a full-length nucleic acid sequence or human interferon-like polypeptide (*see* page 62). The amino

acid sequence of human interferon ike polypeptide was determined from the latter nucleic acid sequence.

4. The dates on the iboratory notebook pages have been redacted from the photocopies. However, the dates e before July 21, 1998, the date on which U.S. Provisional Application No. 60/093,643 was f d, from which U.S. Application No. 09/487,792 claims the benefit of priority, from which U. Patent No. 6,433,145 issued on August 13, 2002.

5. Also accompanyin this Declaration are photocopies of ten (10) pages from a Research Summary showing tha the invention disclosed and claimed in the instant patent application was diligently reduce to practice. Specifically, the photocopies of the Research Summary show that experiments ere performed in order to determine the function of protein encoded by the nucleic acid sequer e described in paragraph 3 above, and that once the function of the protein had been determined, Research Summary was prepared and submitted to the legal department of Amgen Inc., the assi ee of the instant application. More particularly, photocopies of the Research Summary show tha several versions of the human and rat IFN-L proteins were produced in a mammalian expres on system (*see* page 7) and that rat IFN-L:Fc fusion protein treatment of several cell lines wa found to cause phosphorylation of cellular proteins (*see* page 10).

6. The dates on th Research Summary pages have been redacted from the photocopies.

7. We hereby declare urther that all statements made herein by each of us to our own knowledge are true and that all st ements made on information and belief are believed to be true; and further that these statements v re made with the knowledge that willful false statements and the like so made are punishable by fi or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that su willful false statements may jeopardize the validity of the application or any patent issuing ereon.

Dated: June 10, 2004

Signed: Andrew A. Welcher
Andrew A. Welcher

Duanzhi Wen
Duanzhi Wen

Michael Kelly

Michael Kelly

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 99-372-F)

PATENT

In re Application of:	**Welcher et al.**	
Serial No.:	**09/927,850**	**Before the Examiner:** **J. Andres**
Filed:	**August 10, 2001**	**Group Art Unit:** **1646**
For:	**Interferon-Like Molecules and Uses Thereof**	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

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2. The invention disclosed and claimed in the instant patent application was conceived in the United States by us before July 21, 1998 and was then diligently reduced to practice.

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6. The dates on the Research Summary pages have been redacted from the photocopies.

7. We hereby declare further that all statements made herein by each of us to our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: June 25, 2004

Signed: _____
Andrew A. Welcher

_____
Duanzhi Wen

Michael Kelly

ge No.__

[redacted] hrpe3-00078-F6.

① related to rIFNβ (30%).

② ⊕ in Certain Lot of pancreas mRNA(human) Northern (J Cao).

③ Screening of pancreas cDNA (human) Library ⊖.

? Different Lot of RNA source

? Different level of expression

Decision: ① re-Screening human pancreas Library

② determine the genomic screening condition

③ Is genomic screening feasible?

[redacted] IFN probe for genomic blot

17

PAGE: 1

ID: CHERENCOV 0.5 [redacted] 13:16
USER: 2 COMMENT:

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# :YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

GAM NO	POS	TIME MIN	IC#	32P CPM	%ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	655.1	427775.8	0.43	0.00	0.83

4.3×10^5 cpm/λ

d & Understood by me Date

Page No.___

[redacted]

PCR-Hot rIFN probe: template 1λ
1795-01 20pm
1795-02 1λ
10X PCR Buf. 10λ
10mM dNTPs 10λ
^{32}P-dCTP 5λ
25mM Mgcl$_2$ 16λ
Taq 1λ
H$_2$O to 100λ

94°C 30sec, 50°C 30sec 74°C 1min for 40 cycles.

G-50 column purified.

count: 4.3×10^5 cpm/λ.

Determine the hybridization and washing condition for genomic screening.

① very likely the homology of hIFN vs. rIFN is in the neighborhood of 60%.

② The formamide should be between 25% - 30%

. Washing condition should start with gentle wash, then elevate the T wash. Determine a condition in which fragment is detectable while background is minor.

To Page No.____

[redacted]

From the genomic southern (D. Wen keeps all the photo) it is clear:

① there is an 1.8kb HindIII fragment that strongly hybridize w/ probe.

② The formamide concentration can be adjusted to ~30% for relatively optimal signal vs. noice ratio.

③ Washing can be conducted @ ~55°C in 0.2 OR 0.3X SSC, 0.1% SDS.

Wen further found in literature:

① IFNζ one family member is single gene, ie. no intron.

② A lot of pseduo IFN gene as well.

But what about we identify a gene resemble enrpe3-0078-F6, also proved its expressing tissues?

Page No.___

[redacted] Screening h.Pancreas Library w/ Rat Probe

MIN - probe for human pancreas Library screening PAGE: 1

ID:CHERENCOV 0.5 [redacted] 10:24
USER: 2 COMMENT:

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# :YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	606.2	827666.2	0.31	0.01	0.91

PCR as on p. 35.
Library screening
- 1x10^6 clone on 20 plates.
- 30% formamide. 5xSSC. 42°C O/N
- Wash: 2x2xSSC, 0.1% SDS 30min @ 50°C
- O/N exposure

And
There is no double positive clones.

To Page No.____

Read & Understood by me,	Date	Invented by	Date

e No.__

[redacted] Screening human fibroblast genomic Library.

Library : human lung fibroblast genomic Library in FixII vector (Stratagene).

1×10^6 independent clones on 10 Nitro-cellulose membrane.

PCR probe: as described on p.35

-198
IFN probe for genomic Screening

PAGE: 1

[redacted] 12:47

```
D = CHERENCOV  0.5                    COMMENT:
ER: 2
ESET TIME :        0.50
TA CALC   :         CPM  H#    : NO  SAMPLE REPEATS:   1   PRINTER      : STD
UNT BLANK :          NO  IC#   :YES  REPLICATES     :   1   RS232        : OFF
O PHASE   :          NO  AQC   : NO  CYCLE REPEATS  :   1
INTILLATOR:         XTAL LUMEX: NO  LOW SAMPLE REJ:   0
W LEVEL   :          NO  HALF LIFE CORRECTION DATE:       none

OTOPE 1:        32P  %ERROR: 0.00  FACTOR:  1.000000  BKG. SUB:      0

AM  POS   TIME   IC#          32P                    LUMEX  ELAPSED
O          MIN                CPM    %ERROR          %      TIME

1 **-1    0.50 632.0  704931.5    0.34    0.01      0.89
```

Lift Library to Nitrocellulose membrane.

① 5 min denature, 5 min neutralization. 1 Sec 2X SSC

② 80°C bake 1 hr.

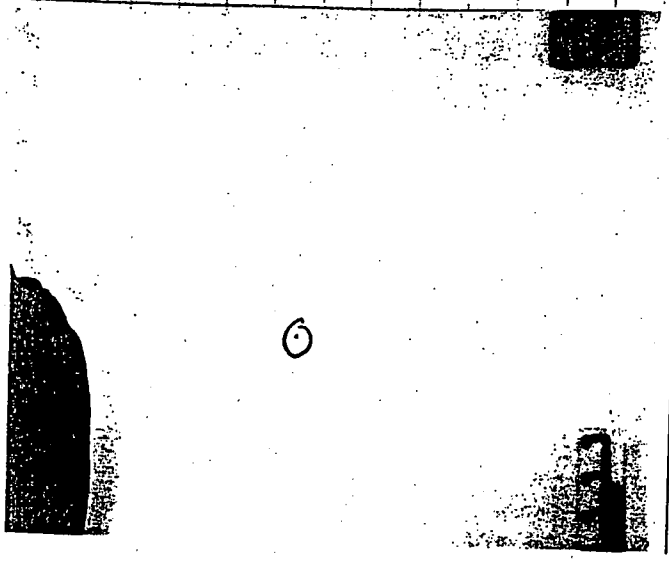
③ pre-hyb: 30% Formamamide
5X SSC
2X Denhart's
100 µg/ml ssDNA
0.1% SDS
2mM EDTA
0.1% Pyrophosphate

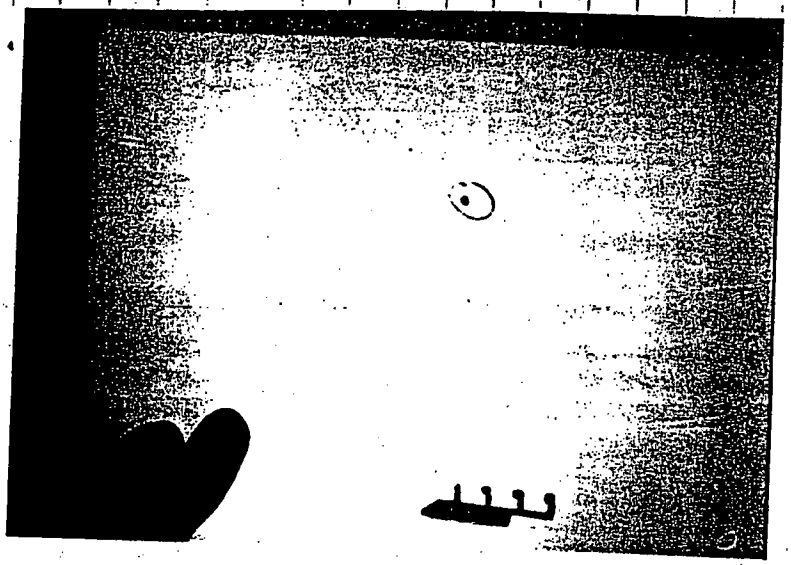
42°C O/N

④ Wash:
At R.T. 1X SSC, 0.1% SDS. 2X 30 min
At 55°C 0.2X SSC, 0.1% SDS 15 min
-80°C BioMax Kodak film O/N at -80°C

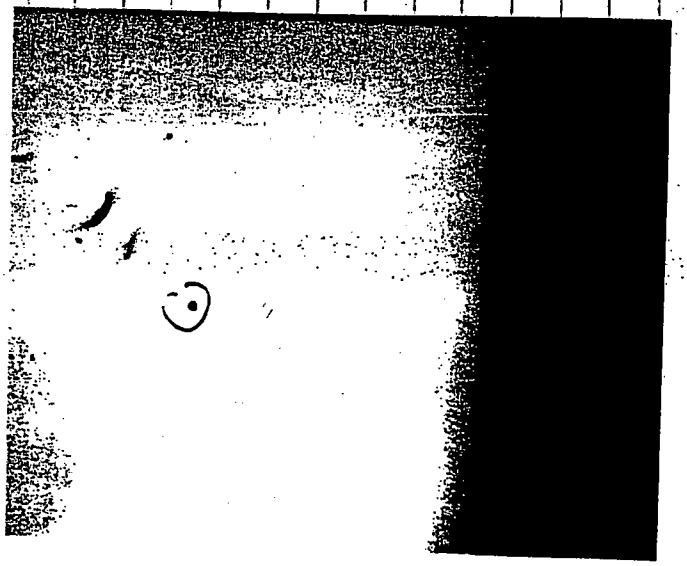
Page No.

[redacted] Primary screening Result.





#2



#6

① pick up the ϕ colony with SM elution 37°C 2hrs.

② 1/500 dilution for secondary

③ double-lift secondary screening membrane.

#7. All double positive

Page No.__

[redacted] 2nd screening > 6 IZH

4/19/98
IZH probe

PAGE: 1

ID: CHERENCOV 0.5 [redacted] 20:13
USER: 2 COMMENT:

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# : YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

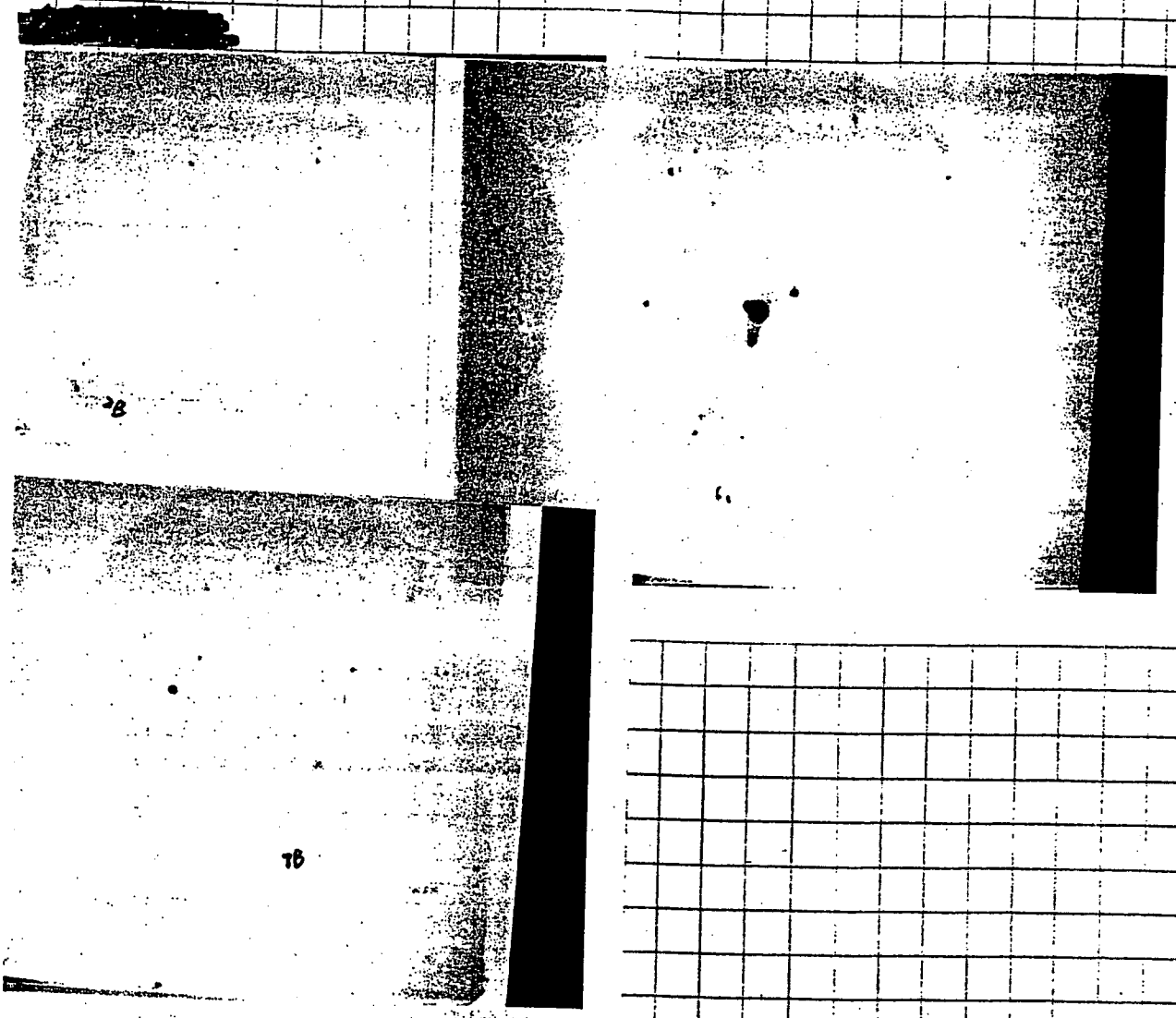
SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	615.7	458314.7	0.42	0.01	0.86

Hybridization: 30% formamide
5xSSC
42°C o/N

Wash: 1xSSC, 0.1% SDS R.T. 30min
0.2xSSC, 0.1%SDS 55°C 15min

To Page No.____

essed & Understood by me,	Date	Invented by	Date



pick-up 2nd clone. elute with SM.
prepare for phage DNA preparation.

n Page No.

[redacted] Φ DNA preparation protocol;

aration of lamdan phage DNA
row up the phage (on plates or in liguid medim).
llecte the supernantat and spin down with 3ooorpn.
dd DNaseI (10u/ml) and RNase(20mg/ml)incubate 30 min at
c degree.
d 1/3 volume 30%PEG 6000 in3M NaCL put in ice for 1 hr or at 4
degree overnight.
10000g centrifugation 20 min
iscard supernatant and interverte the tube on papers to remove
G completely.
d 1/10-1/5 of origin volume T10E1 to suspend the phage
ticles.
d 0.5M EDTA to a final concentration of20mM and proteinaseK
mg/ml ,incubate 1hr in 55c degree waterbath.-
id 5%CATBin 0.5M NaCL to a final concentration of 0.1% and put
65c degree for 5 min.
in down and dissolve in 1.2M NaCL .
Add 2.5vol.of enthanol and spin down.
liscard the enthanol and wash once with 70% enthanol.
.r dry the pellets and dissolve in T10E1 in ice 30 min. then at
ic degree for 10min. don,t put on RT. stock in 4c degree.

- phage has to be amplified once before proceeding to Φ DNA prep. on a 100mm plate. 10µl Φ + top agar → o/n 37°c.
SM elute
- on LB/Agarose plate, grow Φ o/n @ 37°C
- elute w/ SM 2 1/2 hrs @ 7-7.

To Page No.

nessed & Understood by me,	Date	Invented by	Date

e No.__ [redacted]

[redacted]

Human Genomic IFN like clone

BECKMAN DU-600

Φ prep:

Date: [redacted]
Time: 23:19

Leic Acid
adSamples Method SaveClear Print Quit

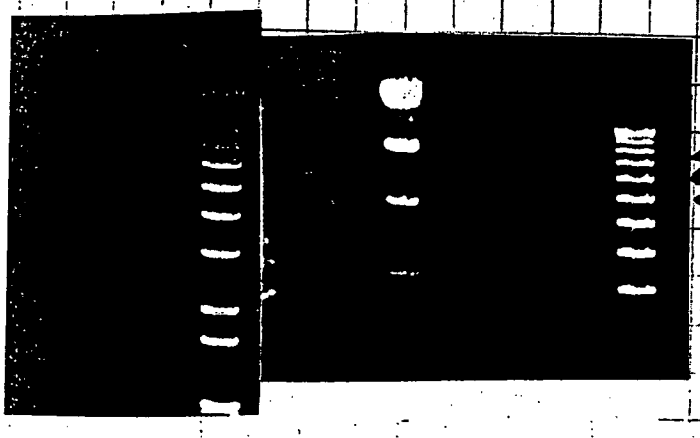
esults file: A:\WORK_RES

Method name: A:\DEFAULT

ssay type: General Ratio and Concentration
ormula setup: VIEW
ampling device: None
ead average time: 0.50 sec

Units: UG/UL
Background Correction: [No]
Concentration: [Yes]
Peak Pick: [No]

mple	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	x100 ssRNA UG/UL	X100 dsDNA UG/UL
#2	0.1428	0.0774	1.8453	0.5419	0.5711	0.7139
#6	0.3206	0.1775	1.8066	0.5535	1.2823	1.6029
#7	0.1731	0.0913	1.8962	0.5274	0.6922	0.8653



#2: 143 ng/λ
#6: 32.1 ng/λ
#7: 17.3 ng/λ

Set-up NotI digestion to release insert in Fix II.

20 mg Φ DNA
10X H
Not I (HC) 40u x 2.5 = 100
H2O

ON @ 37°C incubator.

[redacted] where is the IFNt fragment?

The two ← indicated on p.49 (insert of interest)

Repeat Not I digestion, run a small agarose 0.5% gel.

Transfer and Southern to determine the band

Hyb. 30% formamide, wash: 0.2xSSC, 0.1% SDS.
55°C 15min

Results on p.46

BECKMAN DU-600

Date: [redacted]
Time: 16:18

ucleic Acid
ReadSamples | Method | SaveClear | Print | Quit

Results file: A:\WORK_RES
Method name: A:\DEFAULT

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: None
Read average time: 0.50 sec

Units: UG/UL
Background Correction: [No]
Concentration: [Yes]
Peak Pick: [No]

1/20

Sample ID	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	x100 ssRNA UG/UL	X100 dsDNA UG/UL
1 #2	0.0059	0.0027	2.1876	0.4571	0.0235	0.0293
2 #6	0.0082	0.0043	1.8895	0.5292	0.0326	0.0408
3 #7	0.0085	0.0046	1.8522	0.5399	0.0338	0.0423
4						

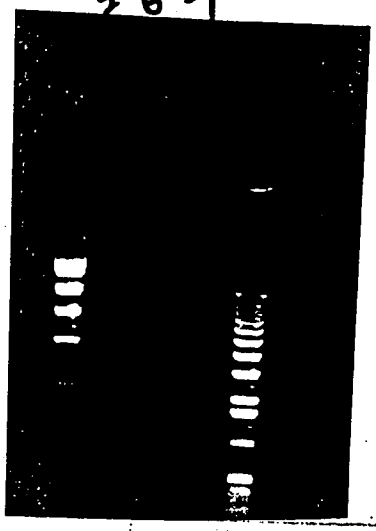
#2 5.9 x 50 x 20 ≈ 6 ng/λ
#6 8.2 x 1000 = 8 ng/λ
#7 8.5 x 1000 = 8.5 ng/λ

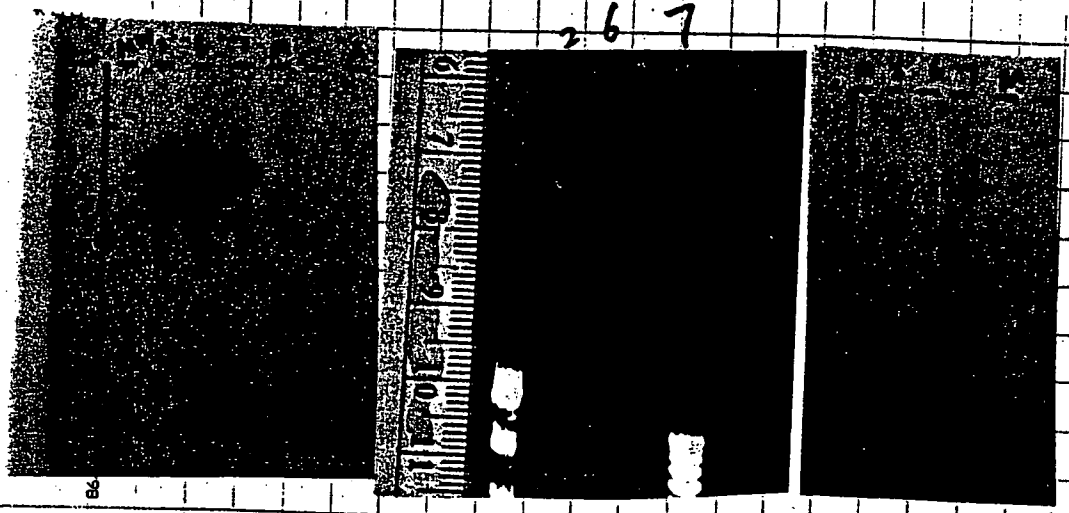
Ligation: 10 ng cut vect.
+ 6 λ Insert
+ 2 λ 10x Buff
5u T4 ligase

essed & Understood by me, | Date | Invented by | Date

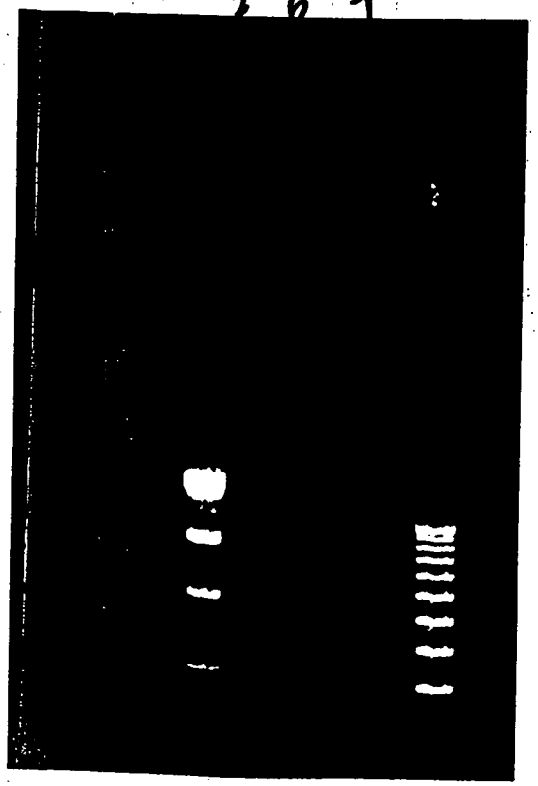
Page No.

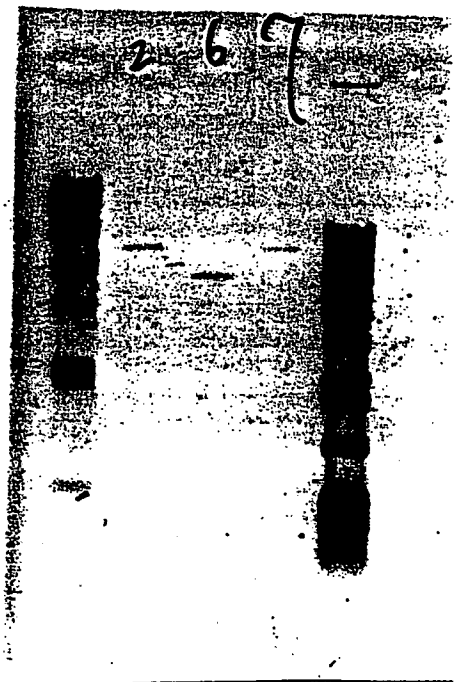
[redacted]





[redacted] Gel purify IFN genomic fragment





d & Understood by me.	Date	Invented by	Date

Page No.

psv.sport/IFN genomic fragment

BECKMAN DU-600

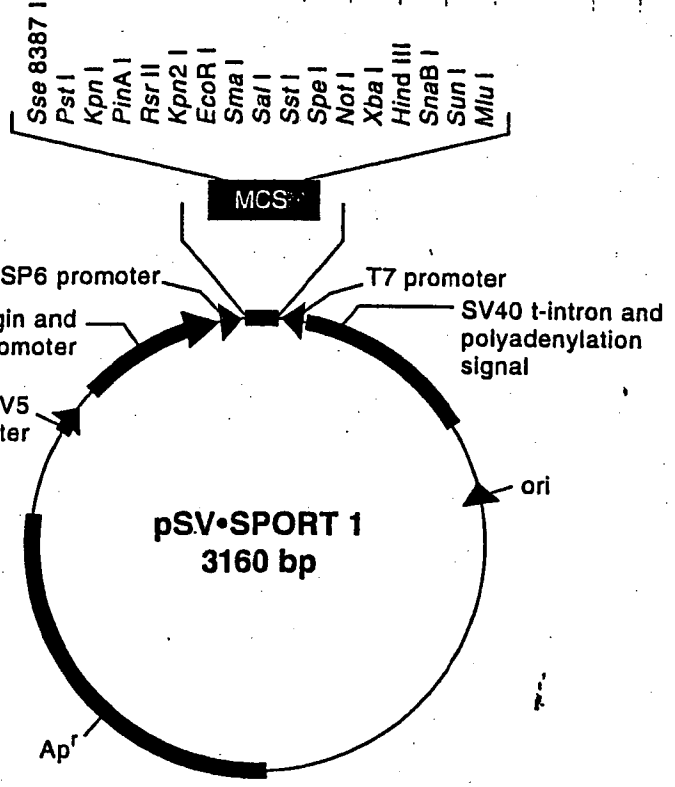
cleic Acid
eadSamples Method

Results file: A:\WORK_RES

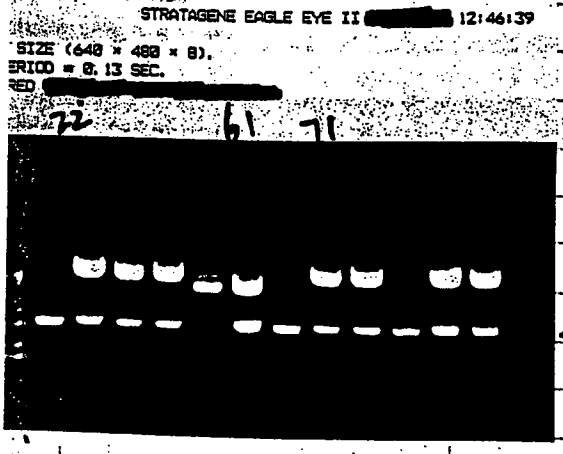
Assay type: General Ratio and Concen
Formula setup: VIEW
Sampling device: None
Read average time: 0.50 sec

Sample ID	abs 260.0 nm	abs 280.0 nm
1 psv.psport HindIII	0.0107	0.0039
2	0.0204	0.0093
3 psv.psport NotI		

psv.psport/NotI 20 ng/λ



0.8% TAE-agarose gel/NotI digestion



miniprep DNA 5μl
10X H 1 μl
NotI 0.5μl (10U)
H2O 3.5μl
Total 10 μl @ 37°C 1½ hrs.

To Page No.

ssed & Understood by me,	Date	Invented by	Date

ge No.__

[redacted] Set-up genomic clone Analysis.

5λ mini-prep DNA
1λ 10x Buffer (BMB)
0.5λ EZ.
3.5λ H2O
10λ @ 37°C 1 1/2 hrs.

8% TAE gel.

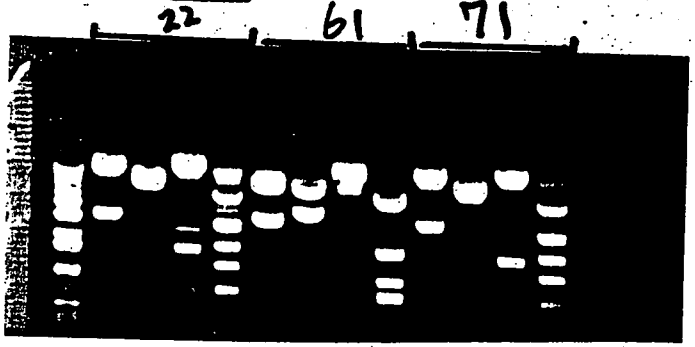
Restriction EZs:
present on MCS of psv.pspart
NotI, EcoRI, XbaI & HindIII

22 and 71 are identical independent clones. but not 61.

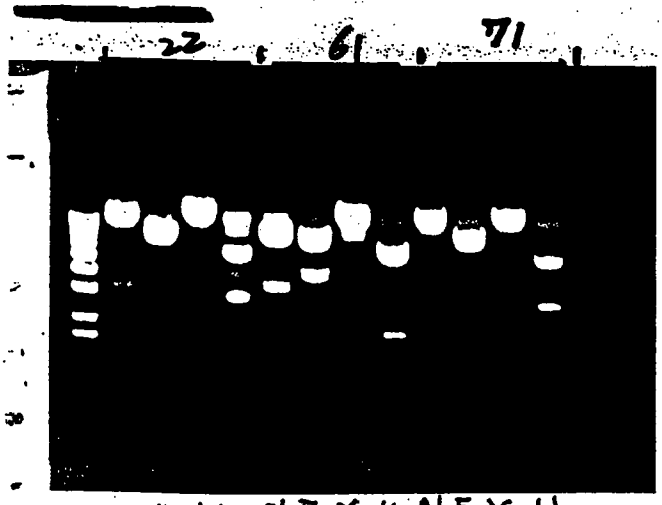
	22	61	71
EcoRI	0	2	0
XbaI	3	2	3
HindIII	>4	>4	>4

Is that why initially EcoRI digestion on genomic DNA didn't light up by rmrep3 probe?

STRATAGENE EAGLE EYE II [redacted] 15:10:42
IE (640 × 480 × 8).
ID = 0.46 SEC.

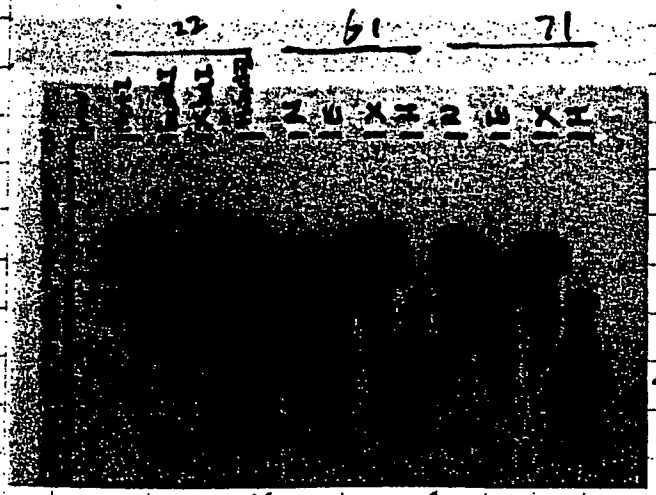


STRATAGENE EAGLE EYE II [redacted] 15:35:05
540 × 480 × 8).
0.66 SEC.



MW EcoRI XbaI HindIII NEXHNEXH

Transfer this gel for alkaline transfer.



* HindIII incomplete digestion

[redacted] Washing Southern Blot.

Hybridization: 30% Formamide, 5x SSC, 2x Denhart's
100µg SSDNA, 0.2% SDS, 2mM EDTA, 0.1% pyrophos
(No NaH_2PO_4)
42°C, 3 hrs. + 5% Dextran Sulfate for Hyb.

PCR-Hot probe: Template 1λ (20ng)
1795-01 1λ (20pm)
1795-02 1λ (20pm)
10mM dNTP 10λ (dCTP@ 0.1mM)
^{32}P-dCTP 5λ
10x PCR Buf. 10λ
25mM $MgCl_2$ 16λ (4mM final)
Taq 1λ
H_2O to 100µl Control: +60µl H_2O
with 10mM dNTPs

5/7/98 rIFN probe

PAGE: 1

ID: CHERENCOV 0.5
USER: 2 COMMENT:
[redacted] 22:04

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# : YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:		none		

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	592.9	528289.8	0.39	0.01	0.86

1ml Count.

Washing: 1 x SSC. 0.1% SDS @ RT 1hr
0.2 x SSC, 0.1% SDS @ 55°C 15min
exp. @ -80°C. O/N.

ssed & Understood by me,	Date	Invented by	Date

le No.___

[redacted] h mrep-3 cloning: Subclone genomic fragment.

Set-up digestion:

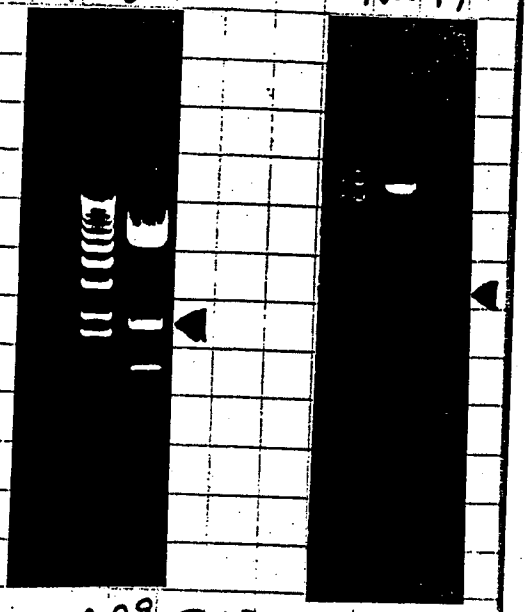
70λ mini-prep DNA
8λ 10x B Buffer (BMB)
2λ BamHI (20u)
80λ total @ 37°C 5 hrs

8λ run gel (with dye)
◀ indicates positive band in Southern Blot with mrep-3 probe (p. 492.

92λ (with dye) run a 8% TAE gel.

Qiagen Gel Purification Kit

No. 61 No. 71



0.8% TAE-agarose gel. (HindIII fragments)

BECKMAN DU-600

Date: [redacted]
Time: 21:25

leic Acid
adSamples Method SaveClear Print Quit

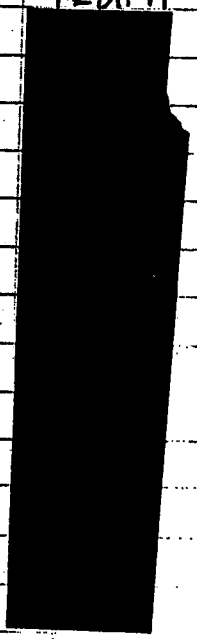
esults file: A:\WORK_RES Method name: A:\DEFAULT

ssay type: General Ratio and Concentration Units: UG/UL
ormula setup: VIEW Background Correction: [No]
ampling device: None Concentration: [Yes]
ead average time: 0.50 sec Peak Pick: [No]

1/10 dilu

ample	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	x100 ssRNA UG/UL	X100 dsDNA UG/UL
#61	0.0168	0.0093	1.7933	0.5576	0.0670	0.0838
#71	0.0201	0.0112	1.7963	0.5567	0.0803	0.1003

No. 61, 71



61, 71 insert is probably same (identical size, HindIII fragment size)

#61 0.0168 × 50 × 20 ≈ 17ng/λ
#71 0.0206 × 50 × 20 ≈ 20ng/λ

& Understood by me. Date Invented by

[redacted] Ligation:

3λ dephosphorylated psv-psport (17ng)
1λ 10x Lig. Butter (BMB)
1λ T4 DNA Ligase (BMB)
5λ Insert (85 ~ 100ng)
10λ

control: +5Nl H2O in vector control.

14°C 1hr

Transformation: 40λ DH10α ElectroMax cell
+ 1.5λ Ligation Mix

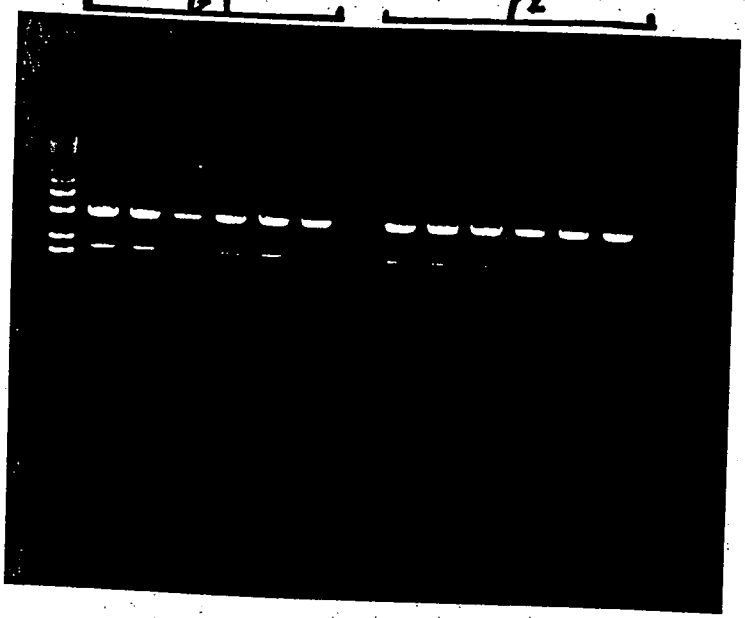
6 transformation clones/each for mini-prep.

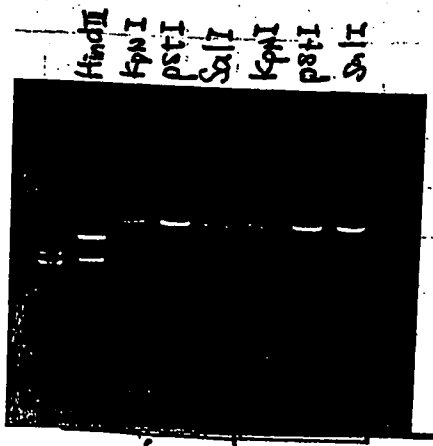
Digest w HindIII
5λ DNA
1λ 10XB (BMB)
0.5λ HindIII (5u)
3.5λ H2O
10λ total 37°C 1½hrs

0.8% TAE gel

STRATAGENE EAGLE EYE II [redacted] 13:20:23
IE SIZE (640 × 480 × 8).
PERIOD = 0.36 SEC.
IRED [redacted]

HindIII

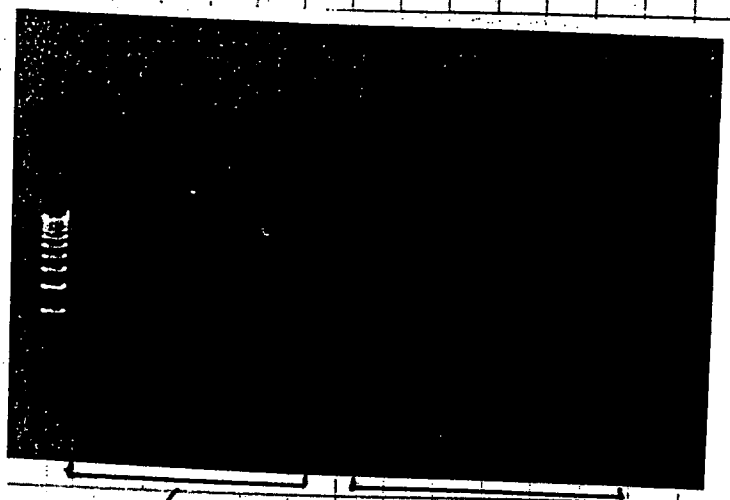




All contain insert. (1.8Kb)

Further Analysis. w
KpNI. pstI. SalI (BMB)
5λ DNA
1λ 10x Buffer
0.5λ EZ (5u)
3.5λ H2O
10λ 37°C 1/2hr

So no additional EZ cut in the insert.



Transfer to N.C. for future reference

61 71

Sequencing Request

ID	Clone	Requestor	Status	Submit	Rcvd
9808902	hgmrep3-6.1	Wen Chen	Pending	[redacted]	00/00/00
9808903	hgmrep3-6.2	Wen Chen	Pending	[redacted]	00/00/00
9808904	hgmrep3-6.5	Wen Chen	Pending	[redacted]	00/00/00
9808905	hgmrep3-7.1	Wen Chen	Pending	[redacted]	00/00/00
9808906	hgmrep3-7.2	Wen Chen	Pending	[redacted]	00/00/00
9808907	hgmrep3-7.2 X deleted!	Wen Chen	Pending	[redacted]	00/00/00
9808908	hgmrep3-7.3	Wen Chen	Pending	[redacted]	00/00/00

3-6.1, 3-6.5, 3-7.1 } identical insert in same orientation

3-6.2, 3-7.2 } identical insert in reverse orientation.

[redacted] mCmp2 Southern: Human Genomic DNA

probe: mCmp2 template 2λ (long)
1582-15' (5' primer) 2λ (20pm)
1536-79C (3' primer) 2λ (20pm)
10x Buffer 10λ
(10mM dNTP; dCTP@0.1mM) 10λ
α-^{32}PdCTP 5λ
25mM $MgCl_2$ 16λ
Taq (BMB) 1λ
H_2O 52λ
100λ

Cold control + 10λ 10mM dNTP + 5λ extra H_2O

94°C 30sec → 60°C 30sec → 72°C 1min → 45 cycles

PAGE: 1

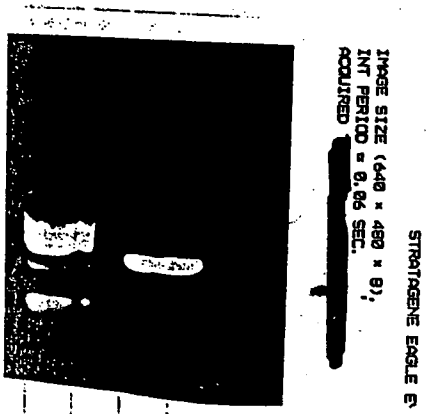
ID: CHERENCOV 0.5
USER: 2 COMMENT:
[redacted] 16:06

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# :YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	602.8	1115564	0.27	0.02	0.95

1λ 1.1 x 10^6 cpm/λ



cold control.

G/ primary Screening:

To Page No. 60

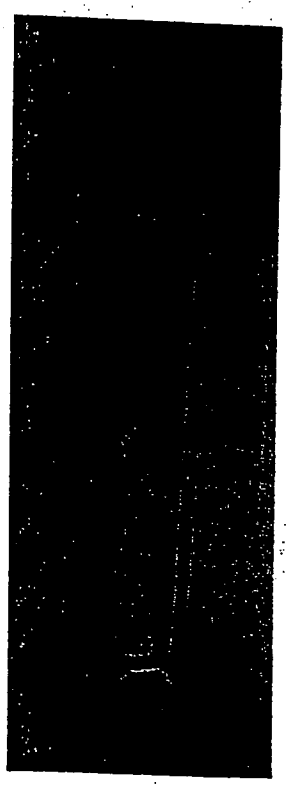
Project No._____
Book No._____ TITLE CMJ2 54

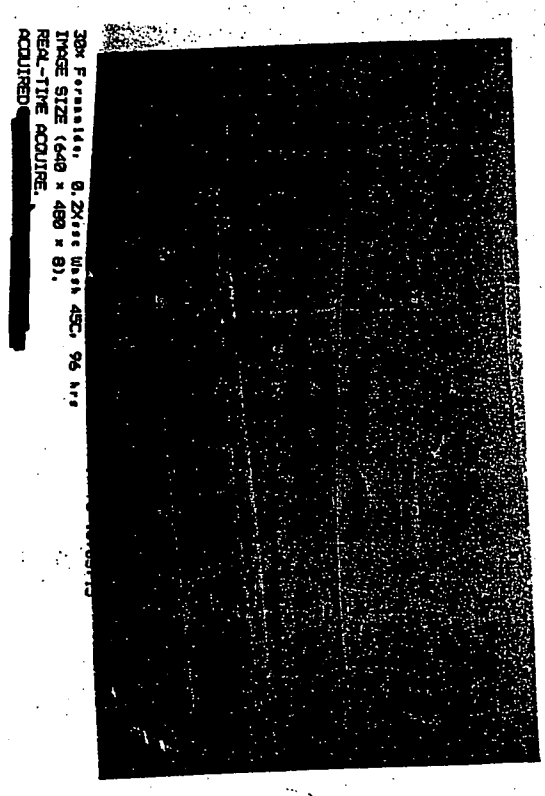
e No.__

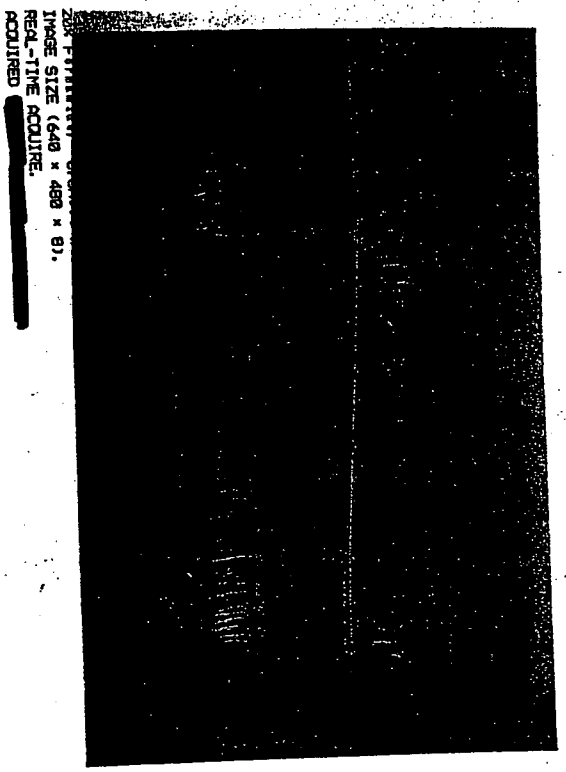
Hybridization in 30% Formamide

	Final	Stock
30% Formamide		~~20~~ 30 ml
5x SSC		25 ml
2x Denhardt's		4 ml
10 MG/ml SS DNA		1 ml
0.2% SDS		1 ml
2mM EDTA		0.4 ml
0.1% Pyrophosphate		1 ml
H2O to 100 ml		37.6 ml

42°C O/N . stripe rehyb.







	30%	20%	20%
ection			
PC (Formamide)			
: SSC	0.2X	0.5X	0.5X
SDS	0.1%	0.1%	0.1%
°C exp	24 hrs	24 hrs	24 hrs

To Page No. 84

REVERSE-COMPLEMENT of: 9808902.Con check: 3852 from: 1 to: 1163
WChen
DNA=hgmrep3-6.1 p=890-24 end
assembled by JK: [redacted]
9808902.con

With 1 enzymes: HINDIII

[redacted] 17:01 ..

```
      CTGAGAAGAGTCACCTGGCAAAATCTGAGACATCTGAGTAGTATGAGCAATTCATTTCCT
    1 ---------+---------+---------+---------+---------+---------+ 60
      GACTCTTCTCAGTGGACCGTTTTAGACTCTGTAGACTCATCATACTCGTTAAGTAAAGGA

a       L  R  R  V  T  W  Q  N  L  R  H  L  S  S  M  S  N  S  F  P  -

      GTAGAATGTCTACGAGAAAACATAGCTTTTGAGTTGCCCCAAGAGTTTCTGCAATACACC
   61 ---------+---------+---------+---------+---------+---------+ 120
      CATCTTACAGATGCTCTTTTGTATCGAAAACTCAACGGGGTTCTCAAAGACGTTATGTGG

a       V  E  C  L  R  E  N  I  A  F  E  L  P  Q  E  F  L  Q  Y  T  -

      CAACCTATGAAGAGGGACATCAAGAAGGCCTTCTATGAAATGTCCCTACAGGCCTTCAAC
  121 ---------+---------+---------+---------+---------+---------+ 180
      GTTGGATACTTCTCCCTGTAGTTCTTCCGGAAGATACTTTACAGGGATGTCCGGAAGTTG

a       Q  P  M  K  R  D  I  K  K  A  F  Y  E  M  S  L  Q  A  F  N  -

      ATCTTCAGCCAACACACCTTCAAATATTGGAAAGAGAGACACCTCAAACAAATCCAAATA
  181 ---------+---------+---------+---------+---------+---------+ 240
      TAGAAGTCGGTTGTGTGGAAGTTTATAACCTTTCTCTCTGTGGAGTTTGTTTAGGTTTAT

a       I  F  S  Q  H  T  F  K  Y  W  K  E  R  H  L  K  Q  I  Q  I  -

      GGACTTGATCAGCAAGCAGAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAAT
  241 ---------+---------+---------+---------+---------+---------+ 300
      CCTGAACTAGTCGTTCGTCTCATGGACTTGGTTACGAACCTCCTTCTGCTCTTACTTTTA

a       G  L  D  Q  Q  A  E  Y  L  N  Q  C  L  E  E  D  E  N  E  N  -

      GAAGACATGAAAGAAATGAAAGAGAATGAGATGAAACCCTCAGAAGCCAGGGTCCCCCAG
  301 ---------+---------+---------+---------+---------+---------+ 360
      CTTCTGTACTTTCTTTACTTTCTCTTACTCTACTTTGGGAGTCTTCGGTCCCAGGGGGTC

a       E  D  M  K  E  M  K  E  N  E  M  K  P  S  E  A  R  V  P  Q  -

      CTGAGCAGCCTGGAACTGAGGAGATATTTCCACAGGATAGACAATTTCCTGAAAGAAAAG
  361 ---------+---------+---------+---------+---------+---------+ 420
      GACTCGTCGGACCTTGACTCCTCTATAAAGGTGTCCTATCTGTTAAAGGACTTTCTTTTC

a       L  S  S  L  E  L  R  R  Y  F  H  R  I  D  N  F  L  K  E  K  -

      AAATACAGTGACTGTGCCTGGGAGATTGTCCGAGTGGAAATCAGAAGATGTTTGTATTAC
  421 ---------+---------+---------+---------+---------+---------+ 480
```

To Page No.

Date

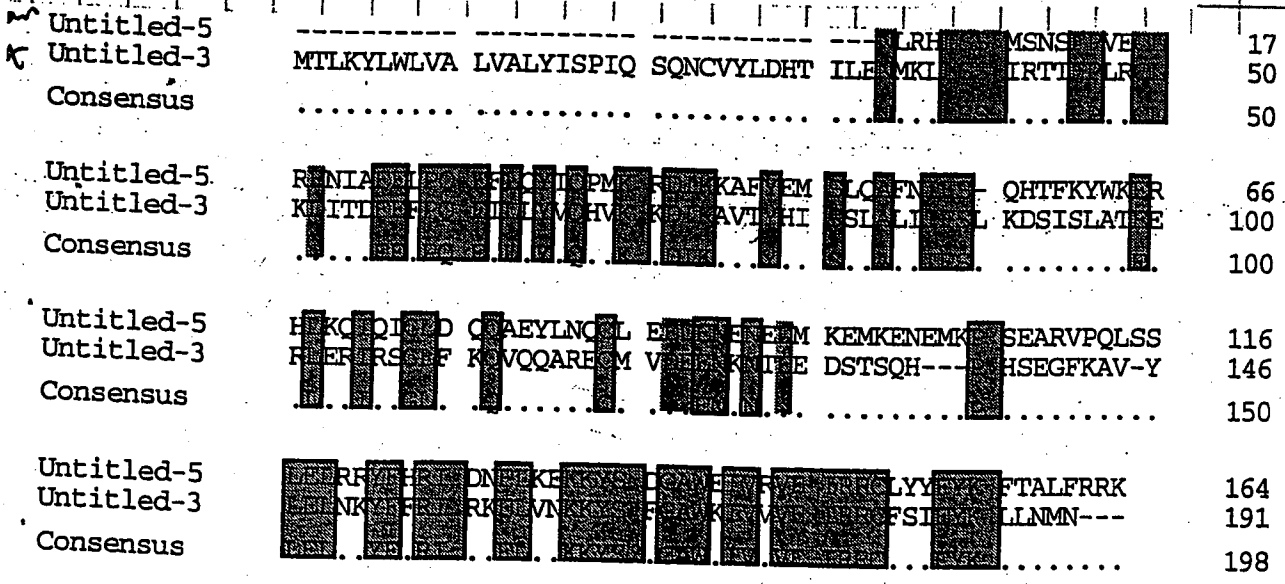
```
    TTTATGTCACTGACACGGACCCTCTAACAGGCTCACCTTTAGTCTTCTACAAACATAATG
    K  Y  S  D  C  A  W  E  I  V  R  V  E  I  R  R  C  L  Y  Y  -
    TTTTACAAATTTACAGCTCTATTCAGGAGGAAATAAGGTATATTTTTGGAATTAAAATTC
481 ---------+---------+---------+---------+---------+---------+ 540
    AAAATGTTTAAATGTCGAGATAAGTCCTCCTTTATTCCATATAAAAACCTTAATTTTAAG
    F  Y  K  F  T  A  L  F  R  R  K  *  G  I  F  L  E  L  K  F  -
    CTTTTCCCTCCGAAATCTCTTTCTCCTTCTCCTCCTCCATCTTCTTTTTAAGGATTGTTG
541 ---------+---------+---------+---------+---------+---------+ 600
    GAAAAGGGAGGCTTTAGAGAAAGAGGAAGAGGAGGAGGTAGAAGAAAAATTCCTAACAAC
    L  F  P  P  K  S  L  S  P  S  P  P  P  S  S  F  *  G  L  L  -
    TGCTGTCCTGTAAGCCTGTCCTCAGTTGGACTGGTAGCCTCGGAACATCAGGGACACTCA
601 ---------+---------+---------+---------+---------+---------+ 660
    ACGACAGGACATTCGGACAGGAGTCAACCTGACCATCGGAGCCTTGTAGTCCCTGTGAGT
    C  C  P  V  S  L  S  S  V  G  L  V  A  S  E  H  Q  G  H  S  -
    CCTCTCTAAGGAGAGGTAATGCCAACCATCCTCAGGGTGACCAAGAGTCTCCTTAGAAAG
661 ---------+---------+---------+---------+---------+---------+ 720
    GGAGAGATTCCTCTCCATTACGGTTGGTAGGAGTCCCACTGGTTCTCAGAGGAATCTTTC
    P  L  *  G  E  V  M  P  T  I  L  R  V  T  K  S  L  L  R  K  -
    TCTTTAAGACATTTTTAAAGGAATAAGATTCCCTCTCCGTCTTCTTCTATTCTCTCTTGC
721 ---------+---------+---------+---------+---------+---------+ 780
    AGAAATTCTGTAAAAATTTCCTTATTCTAAGGGAGAGGCAGAAGAAGATAAGAGAGAACG
    S  L  R  H  F  *  R  N  K  I  P  S  P  S  S  S  I  L  S  C  -
    TCTTTTCTGTGGCCATTTTGAAAGAGCTTTGCTATATATACCACCTGTGGACTTCACCAA
781 ---------+---------+---------+---------+---------+---------+ 840
    AGAAAAGACACCGGTAAAACTTTCTCGAAACGATATATATGGTGGACACCTGAAGTGGTT
    S  F  L  W  P  F  *  K  S  F  A  I  Y  T  T  C  G  L  H  Q  -
    GACAATGGCTAGAGGATAGGGAGCAGAGAATGTTGCAAAATGGTAACATTTCAATGACTT
841 ---------+---------+---------+---------+---------+---------+ 900
    CTGTTACCGATCTCCTATCCCTCGTCTCTTACAACGTTTTACCATTGTAAAGTTACTGAA
    D  N  G  *  R  I  G  S  R  E  C  C  K  M  V  T  F  Q  *  L  -
    AACTGTTTTGCTGCCAAGGTTGCTTATCCTATGAAAATTCAGCACATTAAAAGAGCTTAT
901 ---------+---------+---------+---------+---------+---------+ 960
    TTGACAAAACGACGGTTCCAACGAATAGGATACTTTTAAGTCGTGTAATTTTCTCGAATA
    N  C  F  A  A  K  V  A  Y  P  M  K  I  Q  H  I  K  R  A  Y  -
    ACATGCTCCCTAGAGTCAATACTCTTGCATTTTCCCCCTCCTGCTCGGGGGGAAAAAGGT
961 ---------+---------+---------+---------+---------+---------+ 102
```

To Page No._____

```
a       T C S L E S I L L H F P P P A R G E K G  -

        TGACATTTCTGGCCCATTTCCTTCTCAGCTTGGTTTGTTTGAATTGATGCTTGTGGAATG
1021    ---------+---------+---------+---------+---------+---------+ 1080
        ACTGTAAAGACCGGGTAAAGGAAGAGTCGAACCAAACAAACTTAACTACGAACACCTTAC

INTRON>
INTRON>
a       * H F W P I S F S A W F V * I D A C G M  -

        GTATTTCATTACTTTAAGAGTGAAGATCCATAGTGAAATTGGATGGATGGTTGAATTAGA
1081    ---------+---------+---------+---------+---------+---------+ 1140
        CATAAAGTAATGAAATTCTCACTTCTAGGTATCACTTTAACCTACCTACCAACTTAATCT

a       V F H Y F K S E D P * * N W M D G * I R  -

          HindIII
             |
        CGACCATTAAGCTTACGTACGCG
1141    ---------+---------+--- 1163
        GCTGGTAATTCGAATGCATGCGC

a       R P L S L R T   -
```

Enzymes that do cut:

HindIII



To Page No._____

essed & Understood by me,	Date	Invented by	Date

Page No.___

```
Iomega    MALLFPLLAA LVMTSYSPVG SLGCDLPQNH GLLSRNTLVL LHQMRRISPF   50
I clone   ---------- ---------- -----LRR-- --VTWQNLRH LSSMSNSFPV   21
ensus     .......... .......... .......... .......... ..........   50

Iomega    LCLKDRRDFR FPQEMVKGSQ LQKAHVMSVL HEMLQQIFSL FHTERSSAAW  100
I clone   ELLRENIALE LPQEFLQYTQ PMKRDIKKAF YEMSLQAFNI F-SQHTFKYW   70
ensus     .......... .......... .......... .......... ..........  100

omega     NMTLLDQLHT GLHQQLQHLE TCLLQVVGEG E--------- ---SAGAISS  138
 clone    KERHLKQIQI GLDQQAEYLN QCLEEDENEN EDMKEMKENE MKPSEARVPQ  120
ensus     .......... .......... .......... .......... ..........  150

omega     -PALTLRRYF QGIRVYLKEK KYSDCAWEVV RMEIMKSLFL STNMQERLRS  187
 clone    LSSLELRRYF HRIDNFLKEK KYSDCAWEIV RVEIRRCLYY FYKFTALFRR  170
ensus     .......... .......... .......... .......... ..........  200

omega     KDRDLGSS                                                195
 clone    K-------                                                171
ensus     K.......                                                208
```

```
IFN        MTLKYLWLVA LVALYISPIQ SQNCVYLDH- --TILENLKL LSSIRTTFPL   47
FNomega    MALLFPLLAA LVMTSYSPVG SLGCDLPQNH GLLSRNTLVL LHQMRRISPF   50
FN clone   ---------- ---------- -----LRR-- --VTWQNLRH LSSMSNSFPV   21
nsensus    M.L....L.A LV....SP.. S..C.L...- --....NL.L LSSMR..FP.   50

IFN        FLKKIITDEE FPQEILLYVQ HVKKDSKAVT YHISSLALII FSLKDSISLA   97
FNomega    LCLKDRRDFR FPQEMVKGSQ LQKAHVMSVL HEMLQQIFSL FHTERSSAAW  100
FN clone   ELLRENIALE LPQEFLQYTQ PMKRDIKKAF YEMSLQAFNI F-SQHTFKYW   70
nsensus    .L.K...D.E FPQE.L.Y.Q ..K.D.K.V. YEMS.QAF.I F....S...W  100

IFN        TEERLERIRS GEFKKVLQAR ESMVQEENKN TEEDSTSQH- --PHSEGFKA  144
FNomega    NMTLLDQLHT GLHQQLQHLE TCLLQVVGEG E--------- ---SAGAISS  138
FN clone   KERHLKQIQI GLDQQAEYLN QCLEEDENEN EDMKEMKENE MKPSEARVPQ  120
nsensus    .E..L.QI.. G..QQ.Q.L. .C...ENEN E.........- --PS......  150

IFN        -VYLEENKYF SFRIRKFLVNKKKKYSFCAWKIVAVVEIRRCFSI FYKLLNMN--  191
FNomega    -PALTLRRYF QGIRVYLKEKKYSDCAWEVVRMEIMKSLFL STNMQERLRS  187
FN clone   LSSLELRRYF HRIDNFLKEKKYSDCAWEIVRVEIRRCLYY FYKFTALFRR  170
nsensus    -..L.LRRYF .RIR.FLKEKKYS.CAWEIV.RVEIRRCL.. FYK.....R.  200

IFN        --------                                                191
Nomega     KDRDLGSS                                                195
N. clone   K-------                                                171
nsensus    K-------                                                208
```

Ratio: 2.484 Gaps: 4
Percent Similarity: 63.383 Percent Identity: 63.383

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

9808902.Con x Mrpe3-00078-F6-Wz.Ctg [redacted] 13:52 ..

```
1152 CACCTGGCAAAATCTGAGACATCTGAGTAGTATGAGCAATTCATTTCCTG 1103
     || || | ||||  ||| || |||||| || || || |   | |||||
 159 CATCTTGGAAAACATGAAACTTCTGAGCAGCATCAGGACCACCTTTCCCT 208

1102 TAGAATGTCTACGAGAAAACATAGCTTTTGAGTTGCCCCAAGAGTTTCTG 1053
     ||  ||||||| ||| | ||  | ||||||||| || |||||| |||||
 209 TAAGATGTCTAAAAGATATCACGGATTTTGAGTTTCCTCAAGAGATTCTG 258

1052 CAATACACCCAACCTATGAAGAGGGACATCAAGAAGGCCTTCTATGAAAT 1003
     |  |||  ||| | | |||| | |||||| |||   | |  |||| | ||
 259 CTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACCTATCATAT 308

1002 GTCCCTACAGGCCTTCAACATCTTCAGCC...AACACACCTTCAAATATT 956
      ||    | ||  | |  || ||||| |   || || || ||     .
 309 ATCTTCTCTGGCGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGG 358

 955 GGAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCA 906
      || ||||  || | |  |||  |||  |  ||||||    | ||||
 359 CGACAGAGGAACGCTTGGAACGTATCAGATCGGGACTTTTCAAACAAGTG 408

 905 GAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACAT 856
      || |        | ||| |||  || || |||||  | |      |||
 409 CAGCAAGCTCGAGAGTGCATGGTAGACGAGGAGAACAAGA......ACAC 452

 855 GAAAGAAATGAAAGAGAATGAGATGAAACCCTCAGAAGCCAG.GGTCCCC 807
     | | |      ||   |  | || | | | ||   |  ||| || |  |
 453 GGAGG.......AGGACAGTACATCACAACATCCTCACTCAGAGGGCTTC 495

 806 CAGCTGAGCAGCCTGGAACTGAGGAGATATTTCCACAGGATAGACAATTT 757
      ||     |  ||||||| |||  |  ||||||  ||| ||    || ||
 496 AAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTCAGAATCAGAAAGTT 545

 756 CCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGTCCGAGTGG 707
     ||||  | | ||||||||||||  |||||||||| ||||||||   ||||
 546 CCTGGTAAATAAGAAATACAGTTTCTGTGCCTGGAAGATTGTCGTGGTGG 595

 706 AAATCAGAAGATGTTTGTATTACTTTTACAAATT 673
     |||| ||||||||||   |   |||||||| |
 596 AAATAAGAAGATGTTTCAGTATATTTTACAAACT 629
```

To Page No.

nessed & Understood by me,	Date	Invented by	Date

lo.__

Gaps: 4
Quality: 1170
lity Ratio: 2.484
Similarity: 63.383
Length: 484

N> type 9808902.pair
FIT of reverse of: 9808902.Con check: 3852 from: 1 to: 1163

gmrep3-6.1 p=890-24 end
bled by JK:[redacted]
02.con

Mrpe3-00078-F6-Wz.Ctg check: 4485 from: 1 to: 963

INTRON::DONGYINY [redacted] 16:07:43.44
WCHEN

INTRON::JCAO [redacted] 12:24:38.19
DONGYINY . . .

)l comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Swgapdna.Cmp
heck: 2335

Gap Weight: 50 Average Match: 10.000
Length Weight: 3 Average Mismatch: -9.000

Quality: 1170 Length: 484

Programme	Interferon Like Protein			
Investigator:	Duanzhi Wen			
Animal/Quanti	3 rabbits			
Immunogen:	Interferon like Protein			
Protocol:	MSU Standard			
Prog start date:				
Prog end date:	Ongoing			
Comments:	Give all sera to Duanzhi.			
ANIMAL# PI	[redacted]	50% Titer	[redacted]	50% Titer
#3429 5mls	30mls		30mls	
3432 5mls	30mls		30mls	
#3433 5mls	30mls		30mls	

Programme	Interferon Like Protein			
Investigator:	Duanzhi Wen			
Animal/Quanti	3 rabbits			
Immunogen:	Interferon like Protein			
Protocol:	MSU Standard			
Prog start date:				
Prog end date:	Ongoing			
Comments:	Give all sera to Duanzhi.			
NIMAL# PI	[redacted]	50% Titer	[redacted]	50% Titer
3429 5mls	30mls	1 1500	30mls	1 2000
432 5mls	30mls	1 2500	30mls	1 3000
3433 5mls	30mls	1 5000	30mls	1 10,000
[redacted]	50% Titer			
25mls	> 1 10,000			
25mls	> 1 10,000			
25mls	> 1 10,000			

essed & Understood by me, | Date | Invented by | Date

62

REVERSE-COMPLEMENT of: 9808903.Con check: 57 from: 1 to: 1894
WChen
DNA=hgmrep3-6.2
assembled by JK: [redacted]
9808903.con

1.8 kb Human IFN-like clone seq.

With 1 enzymes: HINDIII

[redacted] 15:37 ..

HindIII

```
      CGCGTACGTAAGCTTAATTTAACAAAATTGGAAAAACCTAAACTATACTGTGCTCTGGTG
    1 ---------+---------+---------+---------+---------+---------+ 60
      GCGCATGCATTCGAATTAAATTGTTTTAACCTTTTTGGATTTGATATGACACGAGACCAC

      ACCTAGCAATCAAATAATCACAGTCATTTGGTCAATGTCTATGATTAACTCAATGAGACA
   61 ---------+---------+---------+---------+---------+---------+ 120
      TGGATCGTTAGTTTATTAGTGTCAGTAAACCAGTTACAGATACTAATTGAGTTACTCTGT

      GGATGTTTGGCTATAGCACCAGGTACAAAAAATATATTTTCATGAAGGATCACTCCCTCT
  121 ---------+---------+---------+---------+---------+---------+ 180
      CCTACAAACCGATATCGTGGTCCATGTTTTTTATATAAAAGTACTTCCTAGTGAGGGAGA

      TATGTAATAGATTTGGGTGAGTGAGTGAGTGAGTGAGTGCATGGACTCACAGCTTTTGGC
  181 ---------+---------+---------+---------+---------+---------+ 240
      ATACATTATCTAAACCCACTCACTCACTCACTCACTCACGTACCTGAGTGTCGAAAACCG

      TTTCTGAAATACCCTGCATCAGTCTTGTTATGATGATTCCTTAGTGCTGGGATGGATCAT
  241 ---------+---------+---------+---------+---------+---------+ 300
      AAAGACTTTATGGGACGTAGTCAGAACAATACTACTAAGGAATCACGACCCTACCTAGTA

      CCAGGCATTTAAGGTAACACGATGGTAATTCTTTGCTCATTTTTCAGGGAAAAAAAAAAG
  301 ---------+---------+---------+---------+---------+---------+ 360
      GGTCCGTAAATTCCATTGTGCTACCATTAAGAAACGAGTAAAAAGTCCCTTTTTTTTTTC

      TTATCACTTCCAAAGTCGGCATAGTCACCCGAAGTAAAAAAAAAAAAAAAAAAAAAAGC
  361 ---------+---------+---------+---------+---------+---------+ 420
      AATAGTGAAGGTTTCAGCCGTATCAGTGGGCTTCATTTTTTTTTTTTTTTTTTTTTTCG

      CTCAGAGGCAAAGGAAAGGGGCCGCAACCTTGGTTAACTGTGAAATGACGAATGAGAAAA
  421 ---------+---------+---------+---------+---------+---------+ 480
      GAGTCTCCGTTTCCTTTCCCCGGCGTTGGAACCAATTGACACTTTACTGCTTACTCTTTT

      CTCCTCCTGCTGAAGATATTCAGGTATATAAAGGCACATGAAGGAAAACTCAAAACATCA
  481 ---------+---------+---------+---------+---------+---------+ 540
      GAGGAGGACGACTTCTATAAGTCCATATATTTCCGTGTACTTCCTTTTGAGTTTTGTAGT

      TTGTCATATACACATCTTCTGGATTTTTTAGCTTGCAAAAAAAATGAGCACCAAACCTGA
  541 ---------+---------+---------+---------+---------+---------+ 600
      AACAGTATATGTGTAGAAGACCTAAAAAATCGAACGTTTTTTTTACTCGTGGTTTGGACT
      → Start
      TATGATTCAAAAGTGTTTGTGGCTTGAGATCCTTATGGGTATATTCATTGCTGGCACCCT
  601 ---------+---------+---------+---------+---------+---------+ 660
```

To Page

```
                                                                                ATCCCTGGACTGTAACTTACTGAACGTTCACCTGAGAAGAGTCACCTGGCAAAATCTGAG
661 ---------+---------+---------+---------+---------+---------+---------+ 720
                                                                                TAGGGACCTGACATTGAATGACTTGCAAGTGGACTCTTCTCAGTGGACCGTTTTAGACTC

                                                                                ACATCTGAGTAGTATGAGCAATTCATTTCCTGTAGAATGTCTACGAGAAAACATAGCTTT
721 ---------+---------+---------+---------+---------+---------+---------+ 780
                                                                                TGTAGACTCATCATACTCGTTAAGTAAAGGACATCTTACAGATGCTCTTTTGTATCGAAA

                                                                                TGAGTTGCCCCAAGAGTTTCTGCAATACACCCAACCTATGAAGAGGGACATCAAGAAGGC
781 ---------+---------+---------+---------+---------+---------+---------+ 840
                                                                                ACTCAACGGGGTTCTCAAAGACGTTATGTGGGTTGGATACTTCTCCCTGTAGTTCTTCCG

                                                                                CTTCTATGAAATGTCCCTACAGGCCTTCAACATCTTCAGCCAACACACCTTCAAATATTG
841 ---------+---------+---------+---------+---------+---------+---------+ 900
                                                                                GAAGATACTTTACAGGGATGTCCGGAAGTTGTAGAAGTCGGTTGTGTGGAAGTTTATAAC

                                                                                GAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCAGAGTACCTGAA
901 ---------+---------+---------+---------+---------+---------+---------+ 960
                                                                                CTTTCTCTCTGTGGAGTTTGTTTAGGTTTATCCTGAACTAGTCGTTCGTCTCATGGACTT

                                                                                CCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACATGAAAGAAATGAAAGAGAATGA
961 ---------+---------+---------+---------+---------+---------+---------+ 1020
                                                                                GGTTACGAACCTCCTTCTGCTCTTACTTTTACTTCTGTACTTTCTTTACTTTCTCTTACT

                                                                                GATGAAACCCTCAGAAGCCAGGGTCCCCCAGCTGAGCAGCCTGGAACTGAGGAGATATTT
1021 ---------+---------+---------+---------+---------+---------+---------+ 1080
                                                                                CTACTTTGGGAGTCTTCGGTCCCAGGGGGTCGACTCGTCGGACCTTGACTCCTCTATAAA

                                                                                CCACAGGATAGACAATTTCCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGT
1081 ---------+---------+---------+---------+---------+---------+---------+ 1140
                                                                                GGTGTCCTATCTGTTAAAGGACTTTCTTTTCTTTATGTCACTGACACGGACCCTCTAACA

                                                                                CCGAGTGGAAATCAGAAGATGTTTGTATTACTTTTACAAATTTACAGCTCTATTCAGGAG
1141 ---------+---------+---------+---------+---------+---------+---------+ 1200
                                                                                GGCTCACCTTTAGTCTTCTACAAACATAATGAAAATGTTTAAATGTCGAGATAAGTCCTC
                                                                                          end
                                                                                GAAATAAGGTATATTTTTGGAATTAAAATTCCTTTTCCCTCCGAAATCTCTTTCTCCTTC
1201 ---------+---------+---------+---------+---------+---------+---------+ 1260
                                                                                CTTTATTCCATATAAAAACCTTAATTTTAAGGAAAAGGGAGGCTTTAGAGAAAGAGGAAG

                                                                                TCCTCCTCCATCTTCTTTTTAAGGATTGTTGTGCTGTCCTGTAAGCCTGTCCTCAGTTGG
1261 ---------+---------+---------+---------+---------+---------+---------+ 1320
                                                                                AGGAGGAGGTAGAAGAAAAATTCCTAACAACACGACAGGACATTCGGACAGGAGTCAACC

                                                                                ACTGGTAGCCTCGGAACATCAGGGACACTCACCTCTCTAAGGAGAGGTAATGCCAACCAT
1321 ---------+---------+---------+---------+---------+---------+---------+ 1380
                                                                                TGACCATCGGAGCCTTGTAGTCCCTGTGAGTGGAGAGATTCCTCTCCATTACGGTTGGTA

                                                                                CCTCAGGGTGACCAAGAGTCTCCTTAGAAAGTCTTTAAGACATTTTTAAAGGAATAAGAT
1381 ---------+---------+---------+---------+---------+---------+---------+ 1440
                                                                                GGAGTCCCACTGGTTCTCAGAGGAATCTTTCAGAAATTCTGTAAAAATTTCCTTATTCTA

                                                                                TCCCTCTCCGTCTTCTTCTATTCTCTCTTGCTCTTTTCTGTGGCCATTTTGAAAGAGCTT
1441 ---------+---------+---------+---------+---------+---------+---------+ 1500
```

To Page No

Date

```
      AGGGAGAGGCAGAAGAAGATAAGAGAGAACGAGAAAAGACACCGGTAAAACTTTCTCGAA

      TGCTATATATACCACCTGTGGACTTCACCAAGACAATGGCTAGAGGATAGGGAGCAGAGA
1501  ---------+---------+---------+---------+---------+---------+ 1560
      ACGATATATATGGTGGACACCTGAAGTGGTTCTGTTACCGATCTCCTATCCCTCGTCTCT

      ATGTTGCAAAATGGTAACATTTCAATGACTTAACTGTTTTGCTGCCAAGGTTGCTTATCC
1561  ---------+---------+---------+---------+---------+---------+ 1620
      TACAACGTTTTACCATTGTAAAGTTACTGAATTGACAAAACGACGGTTCCAACGAATAGG

      TATGAAAATTCAGCACATTAAAAGAGCTTATACATGCTCCCTAGAGTCAATACTCTTGCA
1621  ---------+---------+---------+---------+---------+---------+ 1680
      ATACTTTTAAGTCGTGTAATTTTCTCGAATATGTACGAGGGATCTCAGTTATGAGAACGT

      TTTTCCCCCTCCTGCTCGGGGGGAAAAAGGTTGACATTTCTGGCCCATTTCCTTCTCAGC
1681  ---------+---------+---------+---------+---------+---------+ 1740
      AAAAGGGGGAGGACGAGCCCCCCTTTTTCCAACTGTAAAGACCGGGTAAAGGAAGAGTCG

      TTGGTTTGTTTGAATTGATGCTTGTGGAATGGTATTTCATTACTTTAAGAGTGAAGATCC
1741  ---------+---------+---------+---------+---------+---------+ 1800
      AACCAAACAAACTTAACTACGAACACCTTACCATAAAGTAATGAAATTCTCACTTCTAGG

                                        HindIII
                                           |
      ATAGTGAAATTGGATGGATGGTTGAATTAGACGACCATTAAGCTTGGATCCTCTAGAGCG
1801  ---------+---------+---------+---------+---------+---------+ 1860
      TATCACTTTAACCTACCTACCAACTTAATCTGCTGGTAATTCGAACCTAGGAGATCTCGC

      GCCGCCGACTAGTGAGCTCGTCGACCCGGGAATT
1861  ---------+---------+---------+---- 1894
      CGGCGGCTGATCACTCGAGCAGCTGGGCCCTTAA
```

Human Clone

ct No. IFN


```
                10                  30                  50
GCGTACGTAAGCTTAATTTAACAAAATTGGAAAAACCTAAACTATACTGTGCTCTGGTG
                70                  90                 110
CCTAGCAATCAAATAATCACAGTCATTTGGTCAATGTCTATGATTAACTCAATGAGACA
               130                 150                 170
GATGTTTGGCTATAGCACCAGGTACAAAAAATATATTTTCATGAAGGATCACTCCCTCT
               190                 210                 230
ATGTAATAGATTTGGGTGAGTGAGTGAGTGAGTGAGTGCATGGACTCACAGCTTTTGGC
               250                 270                 290
TTCTGAAATACCCTGCATCAGTCTTGTTATGATGATTCCTTAGTGCTGGGATGGATCAT
               310                 330                 350
CAGGCATTTAAGGTAACACGATGGTAATTCTTTGCTCATTTTTCAGGGAAAAAAAAAAG
               370                 390                 410
TATCACTTCCAAAGTCGGCATAGTCACCCGAAGTAAAAAAAAAAAAAAAAAAAAAGC
               430                 450                 470
TCAGAGGCAAAGGAAAGGGGCCGCAACCTTGGTTAACTGTGAAATGACGAATGAGAAAA
               490                 510                 530
TCCTCCTGCTGAAGATATTCAGGTATATAAAGGCACATGAAGGAAAACTCAAAACATCA
               550                 570                 590
TGTCATATACACATCTTCTGGATTTTTTAGCTTGCAAAAAAAATGAGCACCAAACCTGA
               610                 630                 650
ATGATTCAAAAGTGTTTGTGGCTTGAGATCCTTATGGGTATATTCATTGCTGGCACCCT
MetIleGlnLysCysLeuTrpLeuGluIleLeuMetGlyIlePheIleAlaGlyThrLe
               670                 690                 710
TCCCTGGACTGTAACTTACTGAACGTTCACCTGAGAAGAGTCACCTGGCAAAATCTGAG
SerLeuAspCysAsnLeuLeuAsnValHisLeuArgArgValThrTrpGlnAsnLeuAr
               730                 750                 770
CATCTGAGTAGTATGAGCAATTCATTTCCTGTAGAATGTCTACGAGAAAACATAGCTTT
HisLeuSerSerMetSerAsnSerPheProValGluCysLeuArgGluAsnIleAlaPh
               790                 810                 830
GAGTTGCCCCAAGAGTTTCTGCAATACACCCAACCTATGAAGAGGGACATCAAGAAGGC
GluLeuProGlnGluPheLeuGlnTyrThrGlnProMetLysArgAspIleLysLysAl
               850                 870                 890
TTCTATGAAATGTCCCTACAGGCCTTCAACATCTTCAGCCAACACACCTTCAAATATTG
PheTyrGluMetSerLeuGlnAlaPheAsnIlePheSerGlnHisThrPheLysTyrTr
               910                 930                 950
AAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCAGAGTACCTGAA
LysGluArgHisLeuLysGlnIleGlnIleGlyLeuAspGlnGlnAlaGluTyrLeuAs
               970                 990                1010
CAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACATGAAAGAAATGAAAGAGAATGA
GlnCysLeuGluGluAspGluAsnGluAsnGluAspMetLysGluMetLysGluAsnGl
              1030                1050                1070
ATGAAACCCTCAGAAGCCAGGGTCCCCCAGCTGAGCAGCCTGGAACTGAGGAGATATTT
MetLysProSerGluAlaArgValProGlnLeuSerSerLeuGluLeuArgArgTyrPh
              1090                1110                1130
CACAGGATAGACAATTTCCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGT
HisArgIleAspAsnPheLeuLysGluLysLysTyrSerAspCysAlaTrpGluIleVa
              1150                1170                1190
GGAGTGGAAATCAGAAGATGTTTGTATTACTTTTACAAATTTACAGCTCTATTCAGGAG
lrgValGluIleArgArgCysLeuTyrTyrPheTyrLysPheThrAlaLeuPheArgAr
              1210                1230                1250
AAATAAGGTATATTTTTGGAATTAAAATTCCTTTTCCCTCCGAAATCTCTTTCTCCTTC
ys
              1270                1290                1310
CTCCTCCATCTTCTTTTTAAGGATTGTTGTGCTGTCCTGTAAGCCTGTCCTCAGTTGG
```

```
ACTGGTAGCCTCGGAACATCAGGACACTCACCTCTCTTAAGGAGAGGTAATGCAAACCA
      1390                1410                1430
CCTCAGGGTGACCAAGAGTCTCCTTAGAAGTCTTTAAGACATTTTTAAGGAATAAGAT
      1450                1470                1490
TCCCTCTCCGTCTTCTTCTATTCTCTCTTGCTCTTTTCTGTGGCCATTTTGAAAGAGCTT
      1510                1530                1550
TGCTATATATACCACCTGTGGACTTCACCAAGACAATGGCTAGAGGATAGGGAGCAGAGA
      1570                1590                1610
ATGTTGCAAAATGGTAACATTTCAATGACTTAACTGTTTTGCTGCCAAGGTTGCTTATCC
      1630                1650                1670
TATGAAAATTCAGCACATTAAAAGAGCTTATACATGCTCCCTAGAGTCAATACTCTTGCA
      1690                1710                1730
TTTTCCCCCTCCTGCTCGGGGGAAAAGGTTGACATTTCTGGCCCATTTCCTTCTCAGC
      1750                1770                1790
TTGGTTTGTTTGAATTGATGCTTGTGGAATGGTATTTCATTACTTTAAGAGTGAAGATCC
      1810                1830                1850
ATAGTGAAATTGGATGGATGGTTGAATTAGACGACCATTAAGCTTGGATCCTCTAGAGCG
```

To Page No.

rat clone

```
          10                  30                  50
GTCGACCCACGCGTCCGGGGTGTTGTAGATATTTTTCCTTTGGAAGAAATACTGAGCACC
          70                  90                 110
AAGGCTGAGATGACACTGAAGTATTTATGGCTGGTGGCCCTCGTGGCTCTATACATTTCA
            MetThrLeuLysTyrLeuTrpLeuValAlaLeuValAlaLeuTyrIleSer
         130                 150                 170
CCCATCCAGTCTCAGAACTGTGTGTATCTGGATCATACCATCTTGGAAAACATGAAACTT
ProIleGlnSerGlnAsnCysValTyrLeuAspHisThrIleLeuGluAsnMetLysLeu
         190                 210                 230
CTGAGCAGCATCAGGACCACCTTTCCCTTAAGATGTCTAAAAGATATCACGGATTTTGAG
LeuSerSerIleArgThrThrPheProLeuArgCysLeuLysAspIleThrAspPheGlu
         250                 270                 290
TTTCCTCAAGAGATTCTGCTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACC
PheProGlnGluIleLeuLeuTyrValGlnHisValLysLysAspIleLysAlaValThr
         310                 330                 350
TATCATATATCTTCTCTGGCGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGGCG
TyrHisIleSerSerLeuAlaLeuIleIlePheSerLeuLysAspSerIleSerLeuAla
         370                 390                 410
ACAGAGGAACGCTTGGAACGTATCAGATCGGGACTTTTCAAACAAGTGCAGCAAGCTCGA
ThrGluGluArgLeuGluArgIleArgSerGlyLeuPheLysGlnValGlnGlnAlaArg
         430                 450                 470
GAGTGCATGGTAGACGAGGAGAACAAGAACACGGAGGAGGACAGTACATCACAACATCCT
GluCysMetValAspGluGluAsnLysAsnThrGluGluAspSerThrSerGlnHisPro
         490                 510                 530
CACTCAGAGGGCTTCAAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTCAGAATCAGA
HisSerGluGlyPheLysAlaValTyrLeuGluLeuAsnLysTyrPhePheArgIleArg
         550                 570                 590
AAGTTCCTGGTAAATAAGAAATACAGTTTCTGTGCCTGGAAGATTGTCGTGGTGGAAATA
LysPheLeuValAsnLysLysTyrSerPheCysAlaTrpLysIleValValValGluIle
         610                 630                 650
AGAAGATGTTTCAGTATATTTTACAAACTACTCAACATGAATTGAGAATCATCCAGCTTC
ArgArgCysPheSerIlePheTyrLysLeuLeuAsnMetAsnEnd
         670                 690                 710
AAGCAAGAACTTAGATAGAAGTTGTGACTGCTCAAATGTCCCCAAGAACGCTTGATTCTA
         730                 750                 770
AGGCTATTGCGAGTCTGCTGCTACACACTTCGGACGCAAGACTTTTCAAGGTCAGGGTTC
         790                 810                 830
AAGGCAGTACAGTCAAAGGAAGTCTTATGTTAAGCAAAAGAAAAATTTCAGTGGAAAAGC
         850                 870                 890
TAGCAGAAATGTCAACTTGTCAAAAAAACAACTTATGGATTATGGCATTGACGTTACTAG
         910                 930                 950
CAAAAAAAATAAAACAAAAAAAAACAACAGTCACTAAAAAAAAAAAAAAAAAAGGGCGGC

CGC
```

Nucleotide comparison

JHN

6

WChen
DNA=hgmrep3-6.2
assembled by JK[redacted]
9808903.con

to: Mrpe3-00078-F6-Wz.Ctg check: 4485 from: 1 to: 963

From: INTRON::DONGYINY [redacted] 16:07:43.44
To: WCHEN
CC:
Subj:
From: INTRON::JCAO [redacted] 12:24:38.19
To: DONGYINY . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Swgapdna.Cmp
CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000
Quality:	1457	Length:	634
Ratio:	2.392	Gaps:	6
Percent Similarity:	63.516	Percent Identity:	63.516

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

9808903.Rev x Mrpe3-00078-F6-Wz.Ctg [redacted] 15:04 ..

```
555 TCTTCTGGATTTTTTAGCTT..GCAAAAAAAATGAGCACCAAACCTGATA 602
    | || | ||| ||||  |||  | | ||| | ||||||||||  |||| |
 21 TGTTGTAGATATTTTTCCTTTGGAAGAAATACTGAGCACCAAGGCTGAGA 70

603 TGATTCAAAAGTGTTTGTGGCTTGAGATCCTTATGGGTATATTCATTGCT 652
    |||  |  ||| ||| ||||| | |. ||| ||| | ||| |||| |
 71 TGACACTGAAGTATTTATGGCTGGTGGCCCTCGTGGCTCTATACATTTCA 120

653 GGCACCCTATCCCTGGACTGTAACTTACTGAACGTTCACCTGAGAAGAGT 702
      || ||  || | | |||||            ||  | |||
121 CCCATCCAGTCTCAGAACTGT.............GTGTATCTGGATCATAC 158

703 CACCTGGCAAAATCTGAGACATCTGAGTAGTATGAGCAATTCATTTCCTG 752
    || || | ||||  ||| || |||||| || || || |   | |||||
159 CATCTTGGAAAACATGAAACTTCTGAGCAGCATCAGGACCACCTTTCCCT 208

753 TAGAATGTCTACGAGAAAACATAGCTTTTGAGTTGCCCCAAGAGTTTCTG 802
    ||  ||||||  ||| | ||  | ||||||||| || |||||| |||||
209 TAAGATGTCTAAAAGATATCACGGATTTTGAGTTTCCTCAAGAGATTCTG 258

803 CAATACACCCAACCTATGAAGAGGGACATCAAGAAGGCCTTCTATGAAAT 852
    |  |||  ||| | | |||| | |||||| |||   | |  |||| | ||
259 CTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACCTATCATAT 308
```

To Page N

```
853 GTCCCTACAGGCCTTCAACATCTTCAGCC...AACACACCTTCAAATATT 899
309 ATCTTCTCTGGCGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGG 358

900 GGAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCA 949
359 CGACAGAGGAACGCTTGGAACGTATCAGATCGGGACTTTTCAAACAAGTG 408

950 GAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACAT 999
409 CAGCAAGCTCGAGAGTGCATGGTAGACGAGGAGAACAAGA......ACAC 452

1000 GAAAGAAATGAAAGAGAATGAGATGAAACCCTCAGAAGCCAG.GGTCCCC 1048
453 GGAGG.......AGGACAGTACATCACAACATCCTCACTCAGAGGGCTTC 495

1049 CAGCTGAGCAGCCTGGAACTGAGGAGATATTTCCACAGGATAGACAATTT 1098
496 AAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTCAGAATCAGAAAGTT 545

1099 CCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGTCCGAGTGG 1148
546 CCTGGTAAATAAGAAATACAGTTTCTGTGCCTGGAAGATTGTCGTGGTGG 595

1149 AAATCAGAAGATGTTTGTATTACTTTTACAAATT 1182
596 AAATAAGAAGATGTTTCAGTATATTTTACAAACT 629
```

IFN

Project No.
Book No.

protein sequence comp

BESTFIT of: 9808903.Pep check: 4904 from: 1 to: 201

TRANSLATE of: 9808903.rev check: 8672 from: 602 to: 1205
generated symbols 1 to: 201.
REVERSE-COMPLEMENT of: 9808903.Con check: 57 from: 1 to: 1894
WChen
DNA=hgmrep3-6.2
assembled by JK: [redacted] . . .

to: Mrpe3-00078-F6-Wz.Pep check: 5990 from: 1 to: 192

TRANSLATE of: mrpe3-00078-f6-wz.ctg check: 4485 from: 70 to: 646
generated symbols 1 to: 192.
From: INTRON::DONGYINY [redacted] 16:07:43.44
To: WCHEN
CC:
Subj: . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Blosum62.Cmp
CompCheck: 6430

Gap Weight:	12	Average Match:	2.912
Length Weight:	4	Average Mismatch:	-2.003
Quality:	292	Length:	194
Ratio:	1.570	Gaps:	3
Percent Similarity:	49.730	Percent Identity:	40.541

Match display thresholds for the alignment(s):
| = IDENTITY
: = 2
. = 1

9808903.Pep x Mrpe3-00078-F6-Wz.Pep [redacted] 14:55 ..

```
      1 MIQKCLWLEILMGIFIAGILSLDCNLLNVHLRRVIWQNLRHLSSMSNSFP 50
        |  | |||  |. ::|.   | .|    |:|     :|:: |||.  .||
      1 MILKYLWLVALVALYISPIQSQNC....VYLDHTILENMKLLSSIRITFP 46

     51 VECLRENIAFELPQEFLQYIQPMKRDIKKAFYEMSLQAFNIFS.QHTFKY 99
        . ||::   || ||| | | | .|:|||   | .|  |  ||| . .
     47 LRCLKDITDFEFPQEILLYVQHVKKDIKAVTYHISSLALIIFSLKDSISL 96

    100 WKERHLKQIQIGLDQQAEYLNQCLEEDENENEDMKEMKENEMKPSEARVP 149
          |  |..|. || .| :   :|: ::||.| :     :.       |
     97 ATEERLERIRSGLFKQVQQARECMVDEENKNIEEDSTSQHPHSEGFKAV. 145

    150 QLSSLELRRYFHRIDNFLKEKKYSDCAWEIVRVEIRRCLYYFYK 193
          ||| :|| ||  ||  |||| |||.|| |||||   |||
    146 ...YLELNKYFFRIRKFLVNKKYSFCAWKIVVVEIRRCFSIFYK 186
```


tnessed & Understood by me, | Date | Invented by | Date

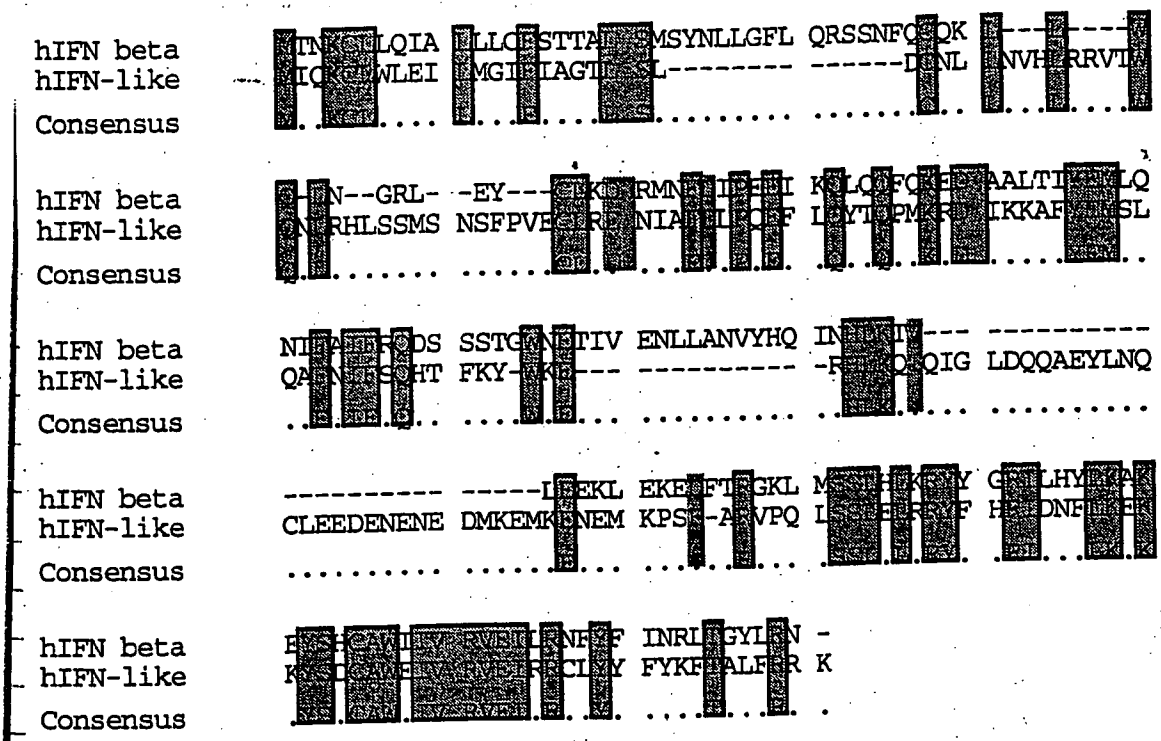
Project No._____

Book No._____ TITLE _____

IFN

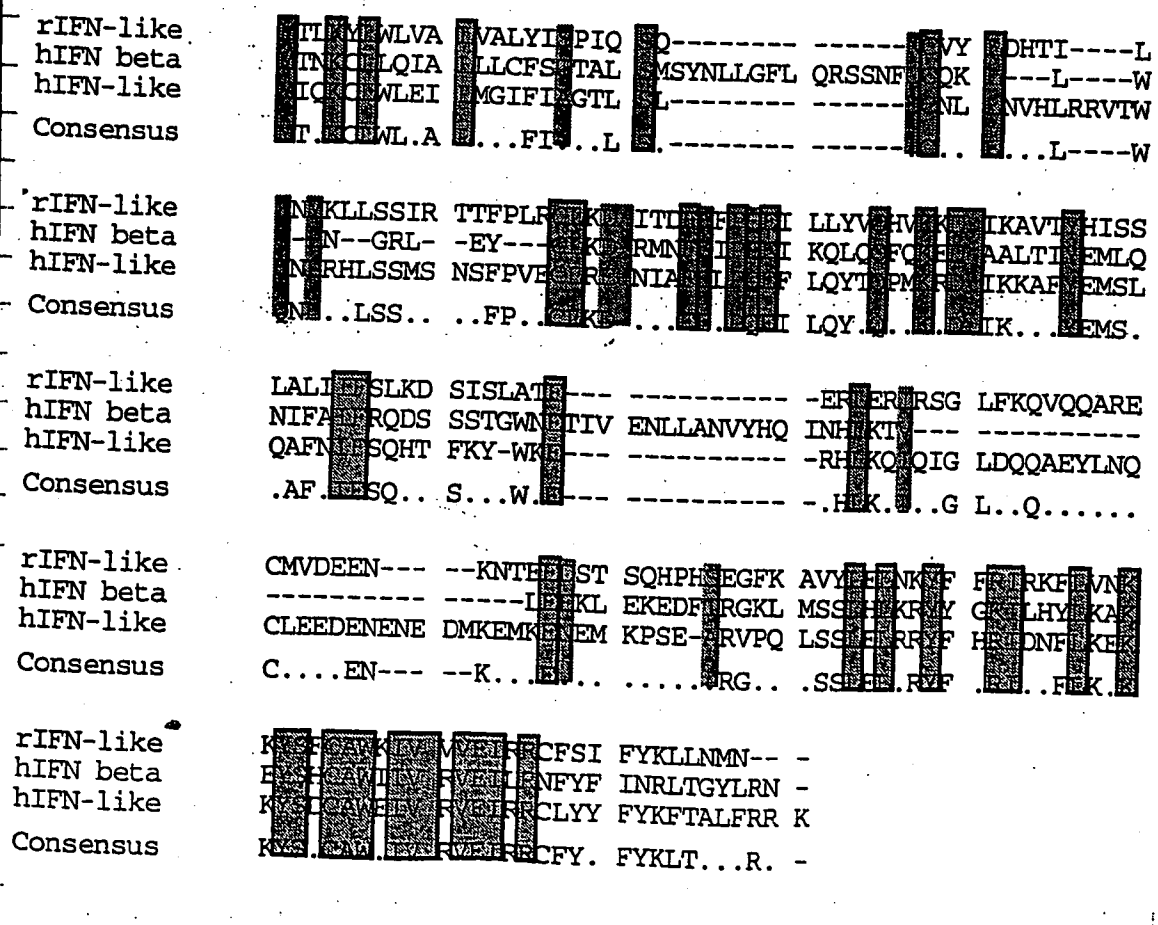
Untitled-5 Formatted Alignment

27% identity



Untitled-5 Formatted Alignment

17% iden



To

71

6/1/98

IFN

```
"IFN"
                 NotI
                 EagI
                 EaeI    EspI   Bsp1286I
                 HhaI    Bpu1102I                                                   AlwNI
                 HinPI BsiEI    BanII                        NspHI      Bsp1286I                      Eam1105I
AAAATCGAGG GCCGCGCGGC CGCTGAGCCC AAATCTTGTG ACAAAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA CCGTCAGTCT   700
TTTTAGCTCC CGGCGCGCCG GCGACTCGGG TTTAGAACAC TGTTTTGAGT GTGTACGGGT GGCACGGGTC GTGGACTTGA GGACCCCCCT GGCAGTCAGA
LysIleGluG lyArgAlaAl aAlaGluPro LysSerCysA spLysThrHi sThrCysPro ProCysProA laProGluLe uLeuGlyGly ProSerValP
   Factor Xa     → Fc  NotI

        10         20         30         40         50         60         70         80         90        100
1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890

      BsiEI
     NotI             Bsp1286I
"IFN" EagI    EspI   BanII                                                    AlwNI
     EaeI    Bpu1102I                         NspHI      Bsp1286I                       Eam1105I
AAAGCGGCCG CTGAGCCCAA ATCTTGTGAC AAAACTCACA CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC CTCTTCCCCC   700
TTTCGCCGGC GACTCGGGTT TAGAACACTG TTTTGAGTGT GTACGGGTGG CACGGGTCGT GGACTTGAGG ACCCCCCTGG CAGTCAGAAG GAGAAGGGGG
LysAlaAlaA laGluProLy sSerCysAsp LysThrHisT hrCysProPr oCysProAla ProGluLeuL euGlyGlyPr oSerValPhe LeuPheProP
   → Fc
```

Oligo No.: 1954-45 Purification: FOP
Researcher: wchen Standard Orde

Sequence

ACG CGT CGA CTT ATT ATT TCC TCC TGA ATA G

Display Expanded Sequence

SalI + 2x stop codon (for pos Rxn)

Oligo No.: 1954-46 Purification: FOP
Researcher: wchen Standard Order

Sequence

AAG GAA AAA AGC GGC CGC TTA TTA TTT CCT CCT GAA TAG AGC

Display Expanded Sequence

NotI + 2x stop codon (for ...)

Oligo No.: 1954-47 Purification: FOPM
Researcher: wchen Standard Order

Sequence

CCC AAG CTT ACC ATG ATT CAA AAG TGT TTG TGG

Display Expanded Sequence

HindIII + ATG @ 5'

Oligo No.: 1954-48 Purification: FOPM
Researcher: wchen Standard Order

Sequence

NotI R G F I — Factor Xa

AAG GAA AAA AGC GGC CGC GCG GCC CTC GAT TT
CCT CCT GAA TAG AGC TGT AA

NotI + FXa + Fc

Display Expanded Sequence

Oligo No.: 1954-49 Purification: FOPM
Researcher: wchen Standard Order

Sequence

NotI

AAG GAA AAA AGC GGC CGC TTT CCT CCT GAA TAG
AGC TGT AA

Display Expanded Sequence

nessed & Understood by me

From Page No.___

Chen, Wen

From: Schultz, Henry
Sent: Friday, [redacted] 5:15 PM
To: Chen, Wen
Subject: RE: human interferon like seq.

Wen - the human predicts cytokine strongly
scoreaacomp= -2.9 scoredipep= 19 ACCEPT (Probablility 91%).

The human is predicted to be signal peptide as follows:

MIQKCLWLEILMGIFIAGTLS cleavage LD.....etc

For the rat, the cytokine prediction is lost but the signal peptide is:

MTLKYLWLVALVALYISPIQS cleavage QN....etc

Henry

From: Chen, Wen
Sent: Friday, [redacted] 4:02 PM
To: Schultz, Henry
Subject: human interferon like seq.

Henry: please help me look at the signal peptide for the human clone sequence.
Thanks.

Wen Chen

Human sequence:
MIQKCLWLEI LMGIFIAGTL SLDCNLLNVH LRRVTWQNLR HLSSMSNSFP

51 VECLRENIAF ELPQEFLQYT QPMKRDIKKA FYEMSLQAFN IFSQHTFKYW

101 KERHLKQIQI GLDQQAEYLN QCLEEDENEN EDMKEMKENE MKPSEARVPQ

151 LSSLELRRYF HRIDNFLKEK KYSDCAWEIV RVEIRRCLYY FYKFTALFRR

201 K

10 30 50
CGCGTACGTAAGCTTAATTTAACAAAATTGGAAAAACCTAAACTATACTGTGCTCTGGTG
70 90 110
ACCTAGCAATCAAATAATCACAGTCATTTGGTCAATGTCTATGATTAACTCAATGAGACA
130 150 170
GGATGTTTGGCTATAGCACCAGGTACAAAAAATATATTTTCATGAAGGATCACTCCCTCT
190 210 230
TATGTAATAGATTTGGGTGAGTGAGTGAGTGAGTGAGTGCATGGACTCACAGCTTTTGGC
250 270 290
TTTCTGAAATACCCTGCATCAGTCTTGTTATGATGATTCCTTAGTGCTGGATGGATCAT
310 330 350
CCAGGCATTTAAGGTAACACGATGGTAATTCTTTGCTCATTTTTCAGGAAAAAAAAAAG
370 390 410
TTATCACTTCCAAAGTCGGCATAGTCACCCGAAGTAAAAAAAAAAAAAAAAAAAAAAAGC
430 450 470
CTCAGAGGCAAAGGAAAGGGGCCGCAACCTTGGTTAACTGTGAAATGACGAATGAGAAAA
490 510 530
CTCCTCCTGCTGAAGATATTCAGGTATATAAAGGCACATGAAGGAAAACTCAAAACATCA
550 570 590
TTGTCATATACACATCTTCTGGATTTTTTTAGCTTGCAAAAAAAATGAGCACCAAACCTGA
MetSerThrLysProAs
610 630 650
TATGATTCAAAAGTGTTTGTGGCTTGAGATCCTTATGGGTATATTCATTGCTGGCACCCT
pMetIleGlnLysCysLeuTrpLeuGluIleLeuMetGlyIlePheIleAlaGlyThrLe
670 690 710
ATCCCTGGACTGTAACTTACTGAACGTTCACCTGAGAAGAGTCACCTGGCAAAATCTGAG
uSerLeuAspCysAsnLeuLeuAsnValHisLeuArgArgValThrTrpGlnAsnLeuAr
730 750 770
ACATCTGAGTAGTATGAGCAATTCATTTCCTGTAGAATGTCTACGAGAAAACATAGCTTT
gHisLeuSerSerMetSerAsnSerPheProValGluCysLeuArgGluAsnIleAlaPh
790 810 830
TGAGTTGCCCCAAGAGTTTCTGCAATACACCCAACCTATGAAGAGGGACATCAAGAAGGC
eGluLeuProGlnGluPheLeuGlnTyrThrGlnProMetLysArgAspIleLysLysAl
850 870 890
CTTCTATGAAATGTCCCTACAGGCCTTCAACATCTTCAGCCAACACACCTTCAAATATTG
aPheTyrGluMetSerLeuGlnAlaPheAsnIlePheSerGlnHisThrPheLysTyrTr
910 930 950
GAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCAGAGTACCTGAA
pLysGluArgHisLeuLysGlnIleGlnIleGlyLeuAspGlnGlnAlaGluTyrLeuAs
970 990 1010
CCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACATGAAAGAAATGAAAGAGAATGA
nGlnCysLeuGluGluAspGluAsnGluAsnGluAspMetLysGluMetLysGluAsnGl
1030 1050 1070
GATGAAACCCTCAGAAGCCAGGGTCCCCCAGCTGAGCAGCCTGGAACTGAGGAGATATTT
uMetLysProSerGluAlaArgValProGlnLeuSerSerLeuGluLeuArgArgTyrPh
1090 1110 1130
CCACAGGATAGACAATTTCCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGT
eHisArgIleAspAsnPheLeuLysGluLysLysTyrSerAspCysAlaTrpGluIleVa
1150 1170 1190
CCGAGTGGAAATCAGAAGATGTTTGTATTACTTTTACAAATTTACAGCTCTATTCAGGAG
lArgValGluIleArgArgCysLeuTyrTyrPheTyrLysPheThrAlaLeuPheArgAr
1210 1230 1250
GAAATAAGGTATATTTTTGGAATTAAAATTCCTTTTCCCTCCGAAATCTCTTTCTCCTTC

To Page No.______

Project No._____
Book No._____ TITLE IFN

From Page No.___

gLysEnd

1270 1290 1310
TCCTCCTCCATCTTCTTTTTAAGGATTGTTGTGCTGTCCTGTAAGCCTGTCCTCAGTTGG
1330 1350 1370
ACTGGTAGCCTCGGAACATCAGGGACACTCACCTCTCTAAGGAGAGGTAATGCCAACCAT
1390 1410 1430
CCTCAGGGTGACCAAGAGTCTCCTTAGAAAGTCTTTAAGACATTTTTAAAGGAATAAGAT
1450 1470 1490
TCCCTCTCCGTCTTCTTCTATTCTCTCTTGCTCTTTTCTGTGGCCATTTTGAAAGAGCTT
1510 1530 1550
TGCTATATATACCACCTGTGGACTTCACCAAGACAATGGCTAGAGGATAGGGAGCAGAGA
1570 1590 1610
ATGTTGCAAAATGGTAACATTTCAATGACTTAACTGTTTTGCTGCCAAGGTTGCTTATCC
1630 1650 1670
TATGAAAATTCAGCACATTAAAAGAGCTTATACATGCTCCCTAGAGTCAATACTCTTGCA
1690 1710 1730
TTTTCCCCCTCCTGCTCGGGGGGAAAAAGGTTGACATTTCTGGCCCATTTCCTTCTCAGC
1750 1770 1790
TTGGTTTGTTTGAATTGATGCTTGTGGAATGGTATTTCATTACTTTAAGAGTGAAGATCC
1810 1830 1850
ATAGTGAAATTGGATGGATGGTTGAATTAGACGACCATTAAGCTTGGATCCTCTAGAGCG
1870 1890
GCCGCCGACTAGTGAGCTCGTCGACCCGGGAATT

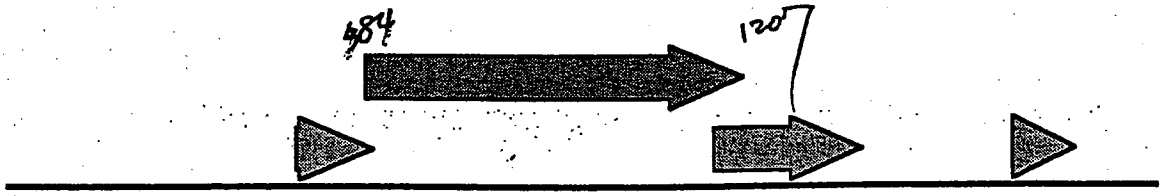
0 400 800 1200 1600 2000

ORF Map (Universal)

Frame 3

Frame 2

Frame 1



Frame -1

Frame -2

Frame -3

One more ATG @ 584 in frame with ATG starting from 602.

To Page N

Witnessed & Understood by me. Date Invented by Date

D 1067
McDonnell
GN-0001

RESEARCH SUMMARY PAGE

Gene Name:	**All Known Alias Gene Names:**
Human: Zhwxc00-00001-a1 Rat: Agp-22423-a1	Member of the interferon family of proteins Name: Interferon-like protein.

Investigator(s):	**Initial Date of Summary Preparation:**
Duanzhi Wen, Andrew Welcher, Michael Kelley	Initial invention disclosure filed [redacted] This summary filled in on [redacted]

Description of Project:

This novel member of the interferon family of proteins is related to the beta, alpha, and omega subfamilies. As an interferon it would be expected to have anti-infective and anti-proliferative uses. Additionally, it might find use in the treatment of multiple sclerosis and other pathologies requiring immunomodulation.

Gene Nucleotide Sequence:

Human:

```
1    CGCGTACGTA AGCTTAATTT AACAAAATTG GAAAAACCTA AACTATACTG
51   TGCTCTGGTG ACCTAGCAAT CAAATAATCA CAGTCATTTG GTCAATGTCT
101  ATGATTAACT CAATGAGACA GGATGTTTGG CTATAGCACC AGGTACAAAA
151  AATATATTTT CATGAAGGAT CACTCCCTCT TATGTAATAG ATTTGGGTGA
201  GTGAGTGAGT GAGTGAGTGC ATGGACTCAC AGCTTTTGGC TTTCTGAAAT
251  ACCCTGCATC AGTCTTGTTA TGATGATTCC TTAGTGCTGG GATGGATCAT
301  CCAGGCATTT AAGGTAACAC GATGGTAATT CTTTGCTCAT TTTTCAGGGA
351  AAAAAAAAAG TTATCACTTC CAAAGTCGGC ATAGTCACCC GAAGTAAAAA
401  AAAAAAAAAA AAAAAAAAGC CTCAGAGGCA AAGGAAAGGG GCCGCAACCT
451  TGGTTAACTG TGAAATGACG AATGAGAAAA CTCCTCCTGC TGAAGATATT
501  CAGGTATATA AAGGCACATG AAGGAAAACT CAAAACATCA TTGTCATATA
551  CACATCTTCT GGATTTTTTA GCTTGCAAAA AAAATGAGCA CCAAACCTGA
601  TATGATTCAA AAGTGTTTGT GGCTTGAGAT CCTTATGGGT ATATTCATTG
651  CTGGCACCCT ATCCCTGGAC TGTAACTTAC TGAACGTTCA CCTGAGAAGA
701  GTCACCTGGC AAAATCTGAG ACATCTGAGT AGTATGAGCA ATTCATTTCC
751  TGTAGAATGT CTACGAGAAA ACATAGCTTT TGAGTTGCCC CAAGAGTTTC
801  TGCAATACAC CCAACCTATG AAGAGGGACA TCAAGAAGGC CTTCTATGAA
851  ATGTCCCTAC AGGCCTTCAA CATCTTCAGC CAACACACCT TCAAATATTG
901  GAAAGAGAGA CACCTCAAAC AAATCCAAAT AGGACTTGAT CAGCAAGCAG
951  AGTACCTGAA CCAATGCTTG GAGGAAGACG AGAATGAAAA TGAAGACATG
1001  AAAGAAATGA AAGAGAATGA GATGAAACCC TCAGAAGCCA GGGTCCCCCA
1051  GCTGAGCAGC CTGGAACTGA GGAGATATTT CCACAGGATA GACAATTTCC
1101  TGAAAGAAAA GAAATACAGT GACTGTGCCT GGGAGATTGT CCGAGTGGAA
1151  ATCAGAAGAT GTTTGTATTA CTTTTACAAA TTTACAGCTC TATTCAGGAG
1201  GAAATAAGGT ATATTTTTGG AATTAAAATT CCTTTTCCCT CCGAAATCTC
1251  TTTCTCCTTC TCCTCCTCCA TCTTCTTTTT AAGGATTGTT GTGCTGTCCT
1301  GTAAGCCTGT CCTCAGTTGG ACTGGTAGCC TCGGAACATC AGGGACACTC
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1351  ACCTCTCTAA GGAGAGGTAA TGCCAACCAT CCTCAGGGTG ACCAAGAGTC
1401  TCCTTAGAAA GTCTTTAAGA CATTTTTAAA GGAATAAGAT TCCCTCTCCG
1451  TCTTCTTCTA TTCTCTCTTG CTCTTTTCTG TGGCCATTTT GAAAGAGCTT
1501  TGCTATATAT ACCACCTGTG GACTTCACCA AGACAATGGC TAGAGGATAG
1551  GGAGCAGAGA ATGTTGCAAA ATGGTAACAT TTCAATGACT TAACTGTTTT
1601  GCTGCCAAGG TTGCTTATCC TATGAAAATT CAGCACATTA AAAGAGCTTA
1651  TACATGCTCC CTAGAGTCAA TACTCTTGCA TTTTCCCCCT CCTGCTCGGG
1701  GGGAAAAAGG TTGACATTTC TGGCCCATTT CCTTCTCAGC TTGGTTTGTT
1751  TGAATTGATG CTTGTGGAAT GGTATTTCAT TACTTTAAGA GTGAAGATCC
1801  ATAGTGAAAT TGGATGGATG GTTGAATTAG ACGACCATTA AGCTTGGATC
1851  CTCTAGAGCG GCCGCCGACT AGTGAGCTCG TCGACCCGGG AATT
```

Rat:

```
   1 GGGTGTTGTA GATATTTTTC CTTTGGAAGA AATACTGAGC ACCAAGGCTG
  51 AGATGACACT GAAGTATTTA TGGCTGGTGG CCCTCGTGGC TCTATACATT
 101 TCACCCATCC AGTCTCAGAA CTGTGTGTAT CTGGATCATA CCATCTTGGA
 151 AAACATGAAA CTTCTGAGCA GCATCAGGAC CACCTTTCCC TTAAGATGTC
 201 TAAAAGATAT CACGGATTTT GAGTTTCCTC AAGAGATTCT GCTGTACGTC
 251 CAGCATGTGA AAAAGGACAT AAAGGCAGTC ACCTATCATA TATCTTCTCT
 301 GGCGCTAATT ATTTTCAGTC TTAAAGACTC CATCTCCCTG GCGACAGAGG
 351 AACGCTTGGA ACGTATCAGA TCGGGACTTT TCAAACAAGT GCAGCAAGCT
 401 CGAGAGTGCA TGGTAGACGA GGAGAACAAG AACACGGAGG AGGACAGTAC
 451 ATCACAACAT CCTCACTCAG AGGGCTTCAA GGCAGTCTAC CTGGAATTGA
 501 ACAAGTATTT CTTCAGAATC AGAAAGTTCC TGGTAAATAA GAAATACAGT
 551 TTCTGTGCCT GGAAGATTGT CGTGGTGGAA ATAAGAAGAT GTTTCAGTAT
 601 ATTTTACAAA CTACTCAACA TGAATTGAGA ATCATCCAGC TTCAAGCAAG
 651 AACTTAGATA GAAGTTGTGA CTGCTCAAAT GTCCCCAAGA ACGCTTGATT
 701 CTAAGGCTAT TGCGAGTCTG CTGCTACACA CTTCGGACGC AAGACTTTTC
 751 AAGGTCAGGG TTCAAGGTAG TACAGTCAAA GGAAGTCTTA TGTTAAGCAA
 801 AAGAAAAATT TCAGTGGAAA AGCTAGCAGA AATGTCAACT TGTCAAAAAA
 851 ACAACTTATG GATTATGGCA TTGACGTTAC TAGCAAAAAA AATAAAACAA
 901 AAAAAAACAA AAA
```

Gene Amino Acid Sequence:

Human:

```
1   MSTKPDMIQK CLWLEILMGI FIAGTLSLDC NLLNVHLRRV TWQNLRHLSS
51   MSNSFPVECL RENIAFELPQ EFLQYTQPMK RDIKKAFYEM SLQAFNIFSQ
101   HTFKYWKERH LKQIQIGLDQ QAEYLNQCLE EDENENEDMK EMKENEMKPS
151   EARVPQLSSL ELRRYFHRID NFLKEKKYSD CAWEIVRVEI RRCLYYFYKF
201   TALFRRK*
```

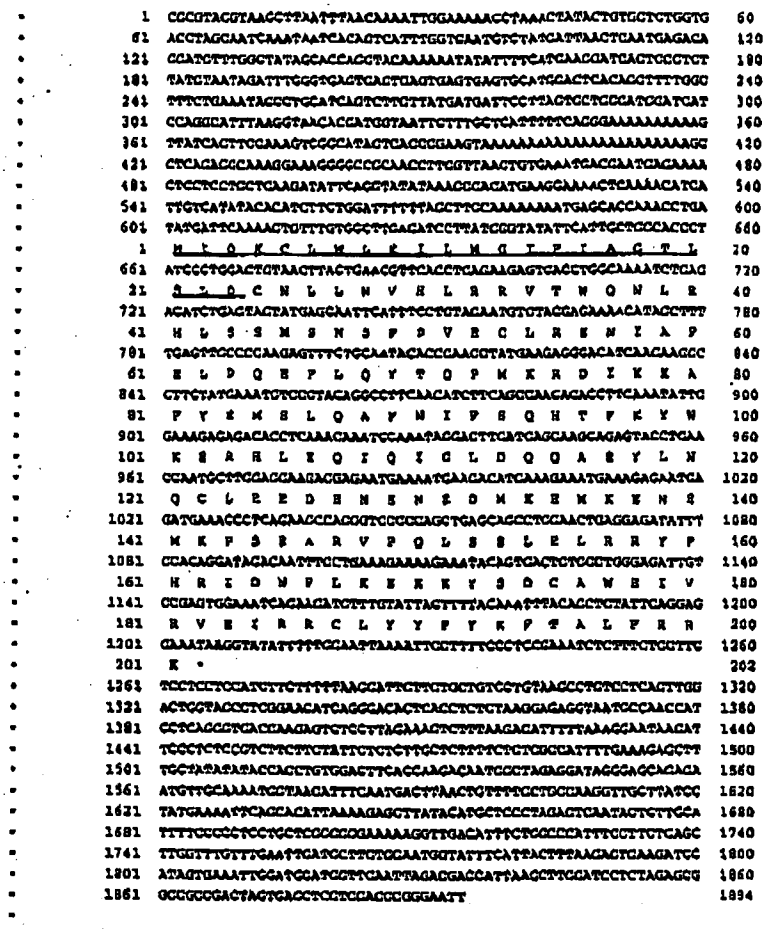
Rat:

```
1   MTLKYLWLVA LVALYISPIQ SQNCVYLDHT ILENMKLLSS IRTTFPLRCL
51   KDITDFEFPQ EILLYVQHVK KDIKAVTYHI SSLALIIFSL KDSISLATEE
101   RLERIRSGLF KQVQQARECM VDEENKNTEE DSTSQHPHSE GFKAVYLELN
151   KYFFRIRKFL VNKKYSFCAW KIVVVEIRRC FSIFYKLLNM N*
```

Figure Containing cDNA and Amino Acid Sequences:

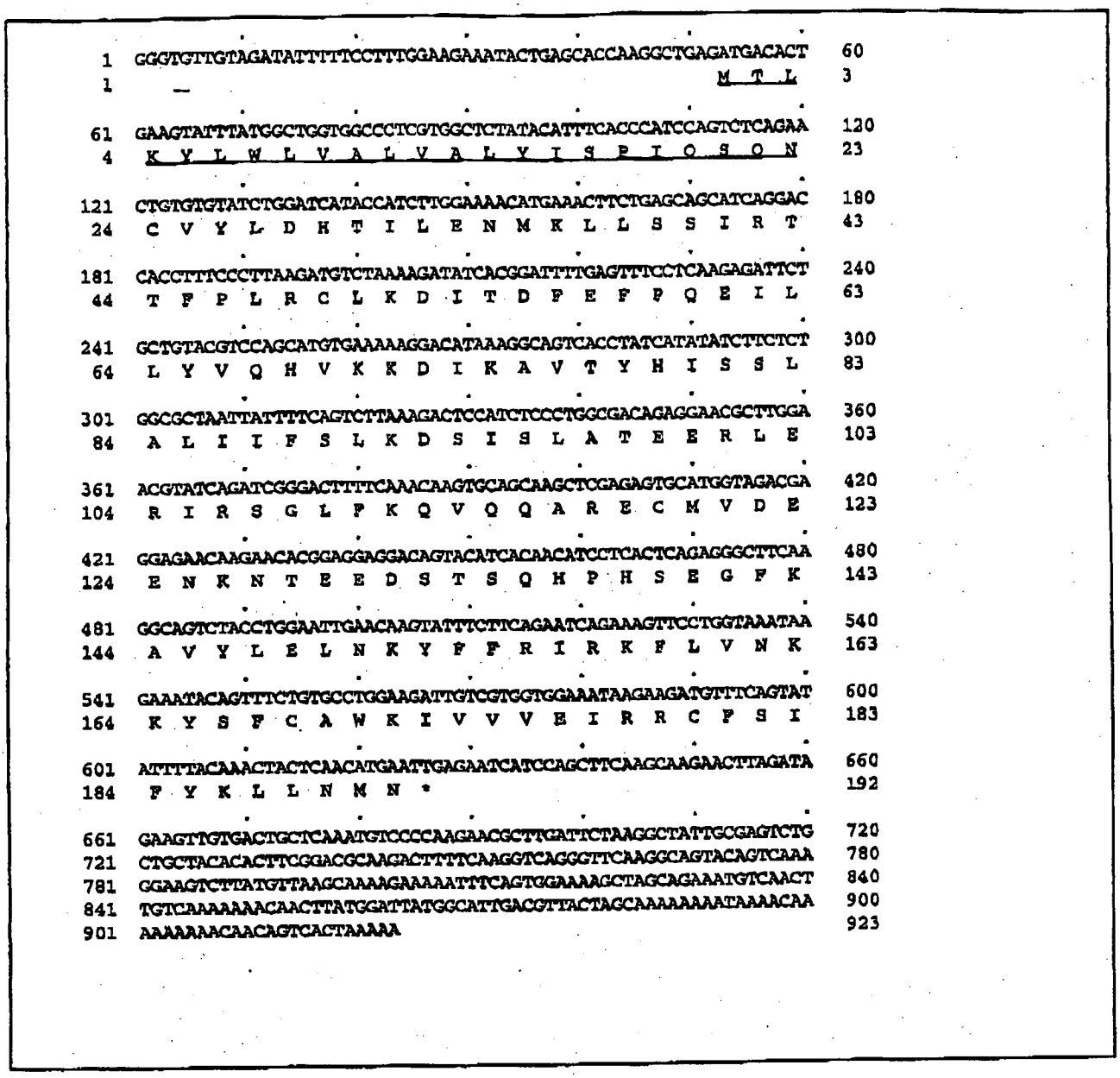
Human:

Sequence Analysis of Human IFN-novel



A human gene which encodes a novel protein of 207 amino acids was isolated by screening the human genomic DNA library using a rat cDNA clone. The deduced amino acid sequence of this novel gene is indicated below the first nucleotide of each codon, and the termination codon is marked with an asterisk. The protein starts with cysteine, and the signal peptide is underlined. This novel protein is 27% identical to human IFN-β.

Rat:



Cloning Information:

The rat sequence was cloned from a rat placenta cDNA library as part of an EST project and was identified by computer analysis as being a novel member of the interferon family of proteins. Briefly, rat embryo day 17 [E17] placenta mRNA was isolated by standard methods (unnecessary information) (Chomczynski, P. and Sacchi, N., Anal. Biochem. 162, 156, 1987). cDNA was synthesized using the SuperScript Plasmid cDNA kit supplied by GIBCO/BRL and subcloned into the pSPORT1 (GIBCO/BRL) vector into the Sal 1 and Not 1 restriction sites.

Cloning of Human IFN-like gene:

Multiple attempts to clone the human IFN-like gene from a variety of human tissue cDNA libraries failed to yield positive clones. However, a human tissue Northern Blot

hybridized with a PCR-generated radioactive rat probe revealed an 1.8 kb Hind III fragment in certain batches of human pancreas mRNA. Attempts to clone this corresponding message in a pancreas cDNA library failed to recover any positive clones.

Examination of the genomic structures of known IFNs revealed that IFN, especially the members in the IFNa family, all share a unique intronless genomic structure. Therefore, screening of human genomic DNA might yield the complete human IFN-like gene. We started with $1x10^6$ human lambda genomic clones (Stratagene, Cat. No. 946206) for primary screening at a density of 50, 000 clonies / plate (*unnecessary information*). Nitrocellulose filters (*unnecessary information*) (S&S) were prepared by standard techniques (Molecular Cloning, A Laboratory Manual, Sambrook, Fritsch, and Maniatis editors).

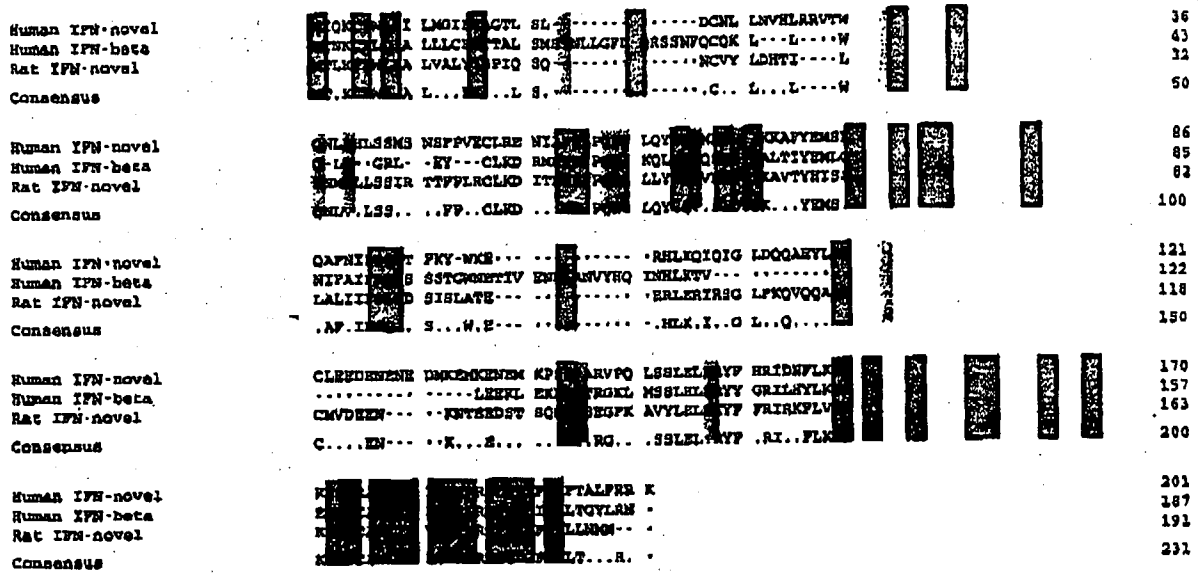
The following conditions were used.

- Prehybridization and hybridization conditions: 30% formamide, 5x SSC, 2x Denhart's, 10μg/ml Salmon sperm DNA, 0.2% SDS, 2mM EDTA and 0.1% pyrophosphate. Hybridization was conducted overnight at 42°C. The washings were done under following conditions: 1x SSC, 0.1%SDS at room temperature for 30-60 minutes followed by 0.2x SSC and 0.1%SDS at 55°C for 15 minutes.
- Generation of radioactive PCR probe (*unnecessary information*): rat cDNA full-length fragment 20ng, primer 1795-01 and 1795-02, 20 pmol each, 1mmol dNTP (dCTP @ 0.01mmol), 32P-dCTP 5 ml and 4mM MgCl2. Reaction condition: denature at 94 °C for 30sec, anneal at 60 °C for 30sec and elongate at 72 °C for 1 minute. The reaction is repeated for a total of 45 times. Simultaneously a "cold" PCR reaction is performed under exact condition except the dNTP mix is dCTP balanced. The radioactive probe was purified by Quick Spin G-50 column and boiled at 100 °C for 10 minutes before chilling on dry ice for 20 minutes. The probe is usually $5x10^5$ cpm/μl.

Three positive clones were recovered after primary, secondary screening and subsequently purified to homogeneity. The lambda phage DNA was prepared by a solid plate culture method. The NotI insert from these clones were excised out and ligated into pSport (GIBCO BRL) vector and transformed into DH10 E. coli strain. The transformants were prepared by Qiagen Spin Column plasmid prep kit. The plasmid DNA was then digested with HindIII. The digested fragments were resolved on agarose gel and transferred to a nylon membrane for Southern Blot analysis. The analysis was conducted under the same condition genomic screening was carried out. The corresponding fragment recognized by "hot" rat probe was then subloned in pSport vector for sequencing analysis. According to the HindIII digestion pattern, we determined these three independent clones were likely to contain identical genomic insert. The sequencing analysis confirmed our speculation. This 1.8kb HindIII fragment contains an open reading frame of 624 base pairs that has 64% similarity to the sequence of rat mrpe3-00078-F6-Wz. In terms of similarity in amino acid sequence, the human sequence is 40.5% identical to and 50% similar to that of rat. All 5 predicted Cysteine residues were perfectly aligned with those in rat protein sequence. Moreover, the human sequence is predicted to contain a signal peptide and cleavage site. The human IFN-like protein is strongly predicted to resemble a secreted cytokine molecule (91% probability).

Homology of Multiple Gene Family Members:

Amino Acid Sequence Alignment of Human IFN-novel, Rat IFN-novel and Human IFN-β



- Human IFN-novel is most close to human IFN-β, with 30% identity. Four out of five cysteine residues are conserved between them.

Presence and Distribution of mRNA in Different Tissues:

Northern blot analysis detected IFN-like mRNA in several different stages of mouse and rat embryos. Northern blots used RNA isolated as above. The full-length rat cDNA was used as a probe. Prehyb conditions were 40 % formamide, 5X SSC, 1 mM EDTA, 0.1 % SDS, for 4 h at 42°C. Hyb conditions were the same as above except were done overnight at 42°C. Blots were washed with 0.2x SSC, 1 mM EDTA, and 0.1% SDS for 30 min at 60°C.

RT-PCR *(conditions are not necessary - standard technology)* identified IFN-like mRNA in the following human tissues : pancreas, small intestine, prostate, uterus, thyroid, and placenta.

Recombinant Protein Expression:

Production of human and rat IFN-like protein in E. coli :

Waiting on data from Karen Sitney. However, the E. coli protein did not appear to be folded correctly and has not yet generated any biologically active material.

Production of human and rat IFN-like protein in a mammalian expression system:

Several versions of the human and rat IFN-like protein have been produced in a mammalian expression system (either CHO or 293 cells). The proteins synthesized were either the native protein itself, or a native protein-Fc fusion. Some of the Fc fusion constructs contained a cleavage site which allows the native protein to be released from the Fc portion after being produced in the conditioned media of CHO cells.

PCR amplification of IFN-like molecule:
PCR primers were selected to amplify the coding sequence of rat/human IFN-like molecule:

Rat IFN-Like Molecule primers:

- IFN-Like molecule Fc-fusion:

1847-77 CCC AAG CTT ACC ATG ACA CTG AAG TAT TTA TG
Forward primer: Hind III site plus ATG
1847-78 AAG GAA AAA AGC GGC CGC ATT CAT GTT GAG TAG

- Reverse primer: Not I site and no stop codon for Fc fusion

Soluble IFN-like molecule:
1896-56 ACG CGT CGA CTC ATC AAT TCA TGT TGA GTA GTT TG
Reverse primer: Sal I site plus 2 stop codons (for pDSRα cloning).
1896-57 AAG GAA AAA AGC GGC CGC TCA TCA ATT CAT GTT GAG TAG
Reverse primer: Not I site plus two stop codons (for pCEP4 cloning).

Human IFN-like primers:

- Soluble human IFN-like primers:

1954-45 ACG CGT CGA CTT ATT ATT TCC TCC TGA ATA G
Reverse prime: Sal I site plus 2 stop codons (for pDSRα cloning).
1954-46 AAG GAA AAA AGC GGC CGC TTA TTA TTT CCT CCT GAA TAG AGC
Reverse primer: Not I site plus two stop codons (for pCEP4 cloning).

- Human IFN like-Fc fusion primers:

1955-44 CCC AAG CTT ACC ATG AGC ACC AAA CCT GAT ATG
Forward primer: Hind III site with 1st ATG
1954-47 CCC AAG CTT ACC ATG ATT CAA AAG TGT TTG TGG C
Forward primer: Hind III site with 2nd ATG
1954-48 AAG GAA AAA AGC GGC CGC ***GCG GCC CTC GAT*** TTT CCT CCT GAA TAG AGC TGT AA
Reverse primer: Not I site, no stop codon with ***Factor Xa*** cleavage site and Fc fusion
1954-49 AAG GAA AAA AGC GGC CGC TTT CCT CCT GAA TAG AGC TGT AA
Reverse primer: Not I site and no stop codon for Fc fusion

PCR Reaction:
Rat:
Reaction Mixture: template 20 ng, 1847-77 and 1847-88 or 1896-56/57, 20 pmol each, 1mmol dNTPs, 4mM MgCl2, 1X PCR buffer, 5u Taq polymerase.
Reaction condition: 2 cycle-linked PCR.
94 °C for 30 °C sec, 50 °C for 30 sec and 72 °C for 1 minute, for a total of 4 cycles, follow by 94

°C for 30 °C sec, 55 °C for 30 sec and 72 °C for 1 minute, for a total of 26 cycles.

Human interferon-like protein PCR conditions:
Reaction Mixture: template 20 ng, 1955-44 and 1954-45or 1954-46 (soluble form) or 1945-48/49 (Fc fusion), 20 pmol each, 1mmol dNTPs, 4mM MgCl2, 1X PCR buffer, 5u Taq plymerase.
Reaction condition: 2 cycle-linked PCR.
94 °C for 30 °C sec, 48 °C for 30 sec and 72 °C for 1 minute, for a total of 4 cycles, follow by 94 °C for 30 °C sec, 55 °C for 30 sec and 72 °C for 1 minute, for a total of 26 cycles.

While 1955-44 primer generates an ORF using first Met in the coding region, a separate PCR with 1954-47 to obtain an insert using 2nd downstream Met was also generated. But in terms of secretion efficiency, when tested in 293 EBNA transient transfection, there was no detectable difference could be defined.

For both rat and human, the PCR products were purified by Qiagen PCR purification spin column and subjected to restriction digestion by respective enzymes (HindIII and NotI (pCEP4) or SalI(pDSRα)). After digestion, the fragment was purified from agarose gel with Qiagen gel purification spin column. The purified fragment was quantified and ligated into pCEP4 (for native form), pCEP4-Fc (for Fc form) or pDSRα (native form or Fc form) vectors respectively. The ligation was transformed into DH10. The transformants were picked for miniprep and subsequent sequencing verification. Accuracy of each cloning fragment was verified by sequencing including the Fc junction sequence. The clone was then maxi-prepared for tissue culture transfection experiments. The IFN-Fc fragment in pCEP4-Fc vector can be released by cutting this vector with HindIII and SalI and re-ligated this fragment into pre-digested pDSRα to yield a vector suitable to transfect CHOD$^-$ cells.

Transfection:

- Protocol for transfeciton into 293 EBNA and CHO cells with lipofectin was adopted from the one used by Jin Cao. Same protocol was used to generate both transient and stable transfectants.
- A commercial available calcium phosphate transfection kit was used in CHO cell stable transfection (protocol is attached).
- A CHO cell transfection and selection protocol from Yi Luo was utilized, except calcium phosphate transfection procedure, which has a commercially available kit.
-

In general, lipofectin transfection yields more stable transfection colonies. Those colonies express comparable level of secreted proteins as those picked from calcium phosphate method.

Generate conditioned media containing recombinant protein.

In order to conduct functional studies on this interferon-like molecule, large quantity of conditioned media (CM) were generated from a pool of hygromycin selected 293 EBNA clones. The cells were cultured in Nunc Triple Flask (500cm) to 80% confluence before switching to serum free media for a week before harvesting. The CM was then sent to purification with protein A affinity chromatography. The purified protein was then used to generate a rabbit polyclonal antibody and to test for in vitro activities. The processing of signal peptide as well as partial amino acid sequence was verified by peptide sequencing.

Purification of human IFN-like-Fc

Conditioned media from CHO cells expressing hulFLM-Fc was thawed and 0.2μm filtered. The filtered material was loaded onto a Protein G column that was previously equilibrated with PBS, pH 7.0 . After loading, the column was washed with PBS until the absorbence at A_{280} reached baseline. The protein was eluted from the column with 0.1M Glycine-HCl pH 2.7 an dimmediately neutralized with 1M Tris-HCl pH 8.5. Fractions containing hulFLM-Fc were pooled and dialyzed into PBS and stored at -70°C.

Factor Xa cleavage of human IFN-like-Fc

The human IFN-like-Fc construct has a Factor Xa cleavage site (IEGR) inserted between the Fc and hulFLM. This site is cleaved with restriction protease factor Xa. The human IFN-like-Fc in PBS was dialyzed into 50mM Tris-HCl, 100mM NaCl, 2mM $CaCl_2$, pH 8.0. The Factor Xa was added to the dialyzed protein at 1/100 (w/w). The sample was incubated overnight at room temperature.

Abs (available, ordered, proposed):

1. Polyclonal:	Polyclonal antibodies were prepared using both rat and human proteins produced in E. coli and CHO cells (from above) using standard immunological techniques. Antisera were positive for the proteins as determined by Western blot analysis (standard techniques)
2. Monoclonal:	None.
3. Peptides:	None.

Phenotype and/or Biological Activity:

1. Transgenic /	(pending / analyzed)

other	
Because the lack of a phenotype constitutes a 'negative' result no conclusions can be drawn from this experiment. Further testing will be required to determine any or all of IFN-like proteins' biological activities in vivo.	
2. *in vivo* assays:	(available, used, proposed)
Not done.	
3. *in vitro* assays:	(available, used, proposed)
Rat IFN-like Fc fusion protein treatment of several cell lines caused phosphorylation of some cellular proteins (unidentified).	

References:

Nothing specifically published on this gene. Lots of references for the interferon family.

Genomic DNA Sequence (i.e. including all introns and exons):

The human gene was cloned from genomic DNA. The attached sequence (above) comes from genomic DNA and includes the coding region which is found in one exon, and the flanking regions.

Ortholog DNA Sequences:

Human and rat sequences cloned.